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БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОИ БИОЛОГИИ И МЕДИЦИНЫ

(BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY)

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

PHYSIOLOGY

THE BLOOD SUPPLY OF THE BRAIN DURING REFLEX REACTIONS

Second Report

THE BLOOD SUPPLY OF THE BRAIN DURING INTEROCEPTIVE IRRITATION OF THE SMALL INTESTINE IN THE PRESENCE OF HYPERCAPNIA

A. M. Blinova

From the Laboratory of the Physiology and Pathology of Respiration and Blood Circulation (Chief – Prof. M. E. Marshak) of the Institute of Normal and Pathological Physiology) (Director – Prof. V. N. Chernigovsky, Active Member of the Academy of Medical Sciences of the USSR) of the Academy of Medical Sciences of the USSR, Moscow

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In the preceding report it was shown that with irritation of the interoceptors of the small intestine in some cases there occurs an increase, and in others a decrease, in the blood supply of the brain. On the basis of an analysis of synchronous changes in the volumetric rate of blood flow and in arterial pressure, the supposition was advanced that the nature of the reaction of the vessels of the brain during interoceptive irritation on the whole depends on the initial tonus of these vessels prior to irritation. For the purpose of verifying experimentally this hypothesis in the present work research was done on the blood supply of the brain during interoceptive irritation in the presence of hypercapnia.

As the data in the literature affirm, the pressure of CO_2 in the blood exerts a considerable influence on the state of tonus of the brain vessels. In a single experiment the pressure of CO_2 in the blood of different animals may vary considerably, depending upon the extent of narcosis, operative procedures and the character of respiration.

It has been established by a series of examinations (for a survey of the literature see the article by Bouckaert and Jordan [5]) that, when the pressure of CO_2 in the blood is increased, as a result of the direct, peripheral effect of CO_2 on the vessel walls there occurs dilation of the vessels and an increase in the supply of blood to the brain, while, when the pressure of CO_2 in the blood falls off, there occurs a constriction of the vessels and a decrease in the brain's blood supply. In contrast to other vascular regions a central vasoconstricting effect of CO_2 is not manifested on the vessels of the brain.

The threshold of the dilating effect of CO_2 for the vessels of the brain proved to be very low. These vessels are dilated at that level of CO_2 in the inspirated air at which neither the respiratory nor the vasomotor center reacts [6, 9, 10 et al].

Some research was devoted to a study of arterial pressure reactions during interoceptive irritation in the presence of short-term hypercapnia. E. Sh. Airapetyants [1], V. N. Zvorykin [2] and T. V. Popova [4] observed during weak hypercapnia (5-7% CO_2 in the mixture) an intensification of the reaction only in those cases in which there was a distinct reaction of respiration and blood pressure in response to inspiration of mixtures containing CO_2. Strong hypercapnia (10% and higher) is accompanied by a two-phase change in the arterial pressure reaction during irritation of the interoceptors: in the initial stages an increase was observed and in the subsequent stages a depression or distortion of the reaction. There is no data in the literature on the change in the blood

supply of the brain during interoceptive irritation in the presence of hypercapnia.

EXPERIMENTAL METHODS

Experiments were conducted on 19 adult dogs under morphine-urethane narcosis.

In accordance with our project we employed low concentrations of CO_2 in the inspirated air, under the influence of which there appeared a distinct increase in the blood supply of the brain and negligible changes in respiration and arterial pressure. For inspiration of the mixtures Douglas bags were used with respiratory valves in a T-pipe inserted in the trachea. Inhalation of the mixture continued for 10-15 minutes.

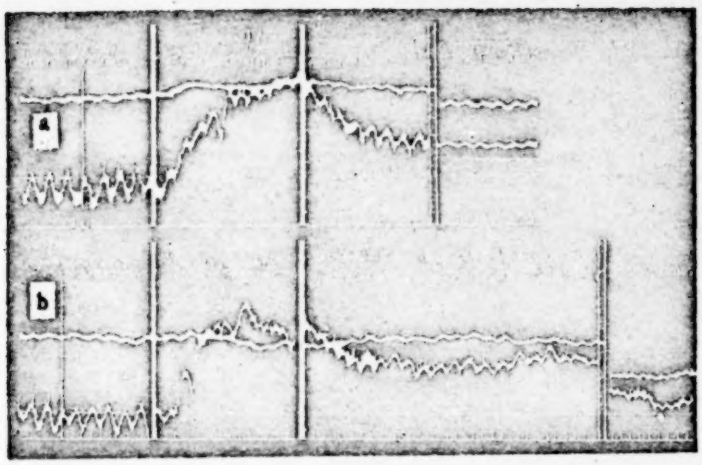

Fig. 1. Photokymogram of the experiment of September 17, 1954. a) Reaction to irritation of the interoceptors during breathing of the air of the room; b) reaction to irritation of the interoceptors 14 minutes after cessation of inhalation of a mixture containing 7.1% CO_2. From above down: respiratory movements, volumetric rate of blood flow in the meninges, arterial pressure, time scale. Vertical lines: the beginning and end of irritation. Double vertical lines: interruption of recording. Broken lines: level of the characteristics being recorded prior to the beginning of irritation.

Irritation of the interoceptors was effected by inflating a section of the small intestine with air under a pressure of 80 mm of mercury. This stimulation was performed 2-3 times until a stable reaction was obtained while the animal breathed the air of the room, once during inhalation of the CO_2 mixture and several times in the course of 1-2 hours after the animal reverted back to breathing the air of the room. The intervals between irritations amounted to not less than 15 minutes. In order to judge the blood supply of the brain we recorded the volumetric rate of blood flow in the meninges by the thermoelectric method using a flat thermoelectrode.

The arterial pressure in the femoral artery was recorded with a mercury manometer and respiratory movements with a pneumograph. All the indicated characteristics were recorded synchronously on a photokymograph.

EXPERIMENTAL RESULTS

During inhalation of the mixtures of air containing 4.5-7.5% CO_2 there was observed a small increase in the amplitude, an alternating increase and decrease in the frequency of respiratory movements and negligible changes in the level of the arterial pressure, which increased as well as decreased within the limits of 5 to 8 mm on a mercury column. At the same time the blood supply of the brain increased after 4-8 seconds and

remained at the higher level throughout the entire period the mixture was inhaled. The significant increase in the volumetric rate of blood flow while arterial pressure was practically unchanged shows that, at the concentrations of CO_2 which we employed, a lowering of tonus occurred, which was manifested by the dilation of the vessels of the brain.

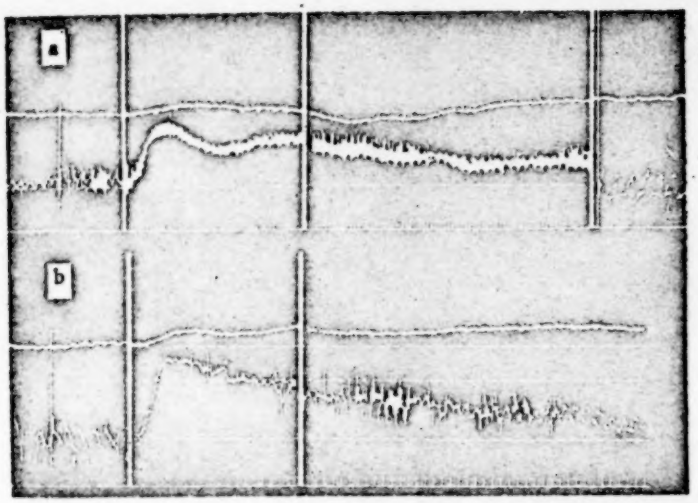

Fig. 2. Photokymogram of the experiment of November 20, 1954. a) Reaction to irritation of the interoceptors during breathing of the air of the room; b) reaction to irritation of the interoceptors during inhalation of a mixture containing 6.9% CO_2. From above down: volumetric rate of blood flow in the meninges, arterial pressure, time scale.

After an animal reverted to breathing the air of the room, within 30-90 seconds the amplitude and frequency of respiratory movements were restored. Arterial pressure, after falling below the initial level for 20-30 seconds, gradually returned to the starting level after 2-3 minutes. Within 10-20 seconds the blood supply of the brain began to decrease, often falling below the starting level, and was restored after 3-8 minutes.

When the room air was breathed, there occurred in all of the experiments in response to irritation of the interoceptors an increase in arterial pressure, in 14 experiments the blood supply of the brain increased (Fig. 1,a), and in 5 experiments a two-phase reaction was observed: an initial increase and a subsequent decrease in blood flow, especially marked in the first few seconds at the conclusion of stimulation (Fig. 2,a). The decrease in blood flow during an increase in arterial pressure indicates constriction of the vessels of the brain.

Irritation of the interoceptors during inhalation of the mixtures containing CO_2 against a background of increased blood supply to the brain led in all of the experiments, regardless of the nature of the original reaction, to an increase in the blood supply of the brain (Fig. 2,b). The blood supply level attained during irritation in the majority of cases was not reduced at the conclusion of stimulation until termination of inhalation of the mixture, although the arterial pressure was restored (Fig. 2,b). In not a single case during inhalation of the CO_2 mixture were phases of reduction of blood flow observed. The reaction with respect to arterial pressure during this time changed negligibly in comparison with the reaction prior to hypercapnia: at times the increase in arterial pressure during irritation of the interoceptors was somewhat greater than, at times somewhat less (± 5-10 mm), and at times the same as in the reaction prior to hypercapnia.

The greater increase and the absence of a reduction phase in the blood supply of the brain during interoceptive irritation in the presence of hypercapnia may be the result of changes in the functional state of the vasomotor center and corresponding impulsation to the vessels of the brain, as well as of changes in the functional state of the vascular wall. Under the conditions of those of our experiments in which small concentrations of

CO_2 were used in the mixtures inhaled, it was hardly possible to assume a decrease in vasoconstricting impulsation from the vasomotor center, inasmuch as the rise in arterial pressure during irritation of the interoceptors was practically the same as the rise prior to hypercapnia. At the same time the decrease in tonus of the vessels of the brain which accompanies hypercapnia, the weakening of the muscular layers of the vascular wall, is linked with the change in functional state of the vessels. On the one hand the weakened wall, under the influence of increased intravascular pressure during irritation of the interoceptors, is stretched more vigorously, and the vascular lumen is passively increased. According to the data of Forbes and his co-workers [6], when normal muscle tonus exists in the vessels of the pia mater, passive dilation of these vessels is negligible or absent even with a very sharp and sudden rise in arterial pressure. When the tonus of these vessels is lowered, their passive dilation is considerable even with a small increase in arterial pressure.

On the other hand, when the vascular wall is weakened, the reactivity of the vessels may change with respect to vasoconstricting influences. Thus H. Rein [10] and H. Rein and U. Otto [11] observed in the inert muscles of the extremities at the normal body temperature of an animal a constriction of the vessels in response to squeezing of the carotid arteries, inhalation of mixtures containing CO_2 and injection of epinephrine. Constriction of the vessels in the presence of these same influences did not occur, however, or was considerably weaker against a background of dilated vessels of the muscles in connection with active hyperemia and when the animal was chilled. The same effect prevailed in response to injection of vasodilating substances. B. Folkow [7] observed during dilation of the vessels of the extremities with adenosinetriphosphate a sharp decrease in the vasoconstricting effect in response to irritation of the sympathetic chain. N. Lüdwigs and M. Schneider [8] did not observe during hypercapnia a decrease in the blood supply of the brain in connection with irritation of the cervical sympathetic nerve, a decrease which was clearly marked prior to hypercapnia.

Proceeding from these data, one must consider that the cause of the change in the reaction of the vessels of the brain resulting from interoceptive irritation during hypercapnia is the lowering of the tonus of the brain vessels in consequence of the peripheral effect on them of the CO_2 in the blood.

The functional state of the vessels of the brain after the animal reverts to breathing the air of the room is restored only gradually, and considerably later than the blood supply of the brain, respiration and arterial pressure. During the first few minutes (from 5 to 10 minutes) after the animal reverted, there was observed during irritation of the interoceptors in all of the experiments an increase in the blood supply of the brain, but it was less marked than before and during hypercapnia, while at the conclusion of the stimulation a prolonged after-effect was not observed; restoration of the original level takes place within the first few seconds.

Subsequently, 20-40 minutes after inhalation of the CO_2 mixture was stopped, in the experiments with an initial two-phase reaction during irritation of the interoceptors, a phase of reduction in blood flow was observed, as it also was prior to hypercapnia. In the majority of experiments with an initial (prior to hypercapnia) single-phase reaction during irritation of the interoceptors there occurred in this period, instead of an increase, a decrease in blood flow (Fig. 2,b), and the initial single-phase reaction was restored only after 60-90 minutes.

Consequently, when the excess carbon dioxide is removed from the blood, the irritability of the vessel walls of the brain to vasoconstricting influences during interoceptive stimulation, before it returns to its original state prior to hypercapnia, passes through a phase of elevation, in consequence of which are created the conditions for lowering the blood flow of the brain in the presence of reflex influences. The data which we obtained show that one of the factors which determine the unequal reaction of the brain vessels during interoceptive irritation is the level of CO_2 pressure in the blood.

SUMMARY

Dynamics of the changes in the blood supply of the brain under the excitation of the interoceptors of the small intestine under usual conditions and under short-term hypercapnia were studied in dogs anesthetized with morphine – urethane. Volumetric velocity of the blood flow in the brain membranes was registered using the thermoelectrical method, as well as the arterial pressure and respiratory movements.

It was noted that during hypercapnia; against a background of the increased blood supply of the brain (owing to the drop of the tonus of the brain vessels), the excitation of the interoceptors brought about a significant increase in the blood supply of the brain in all the cases regardless of the nature of the original reaction before hypercapnia. 60-90 seconds after the termination of the hypercapnia an inversion of the usual reaction was observed; instead of an increase, a decrease of the blood supply of the brain took place. The observed changes of the responses of the brain vessels under the interoceptive excitation during and after hypercapnia are due to the changes in the functional condition of the wall of the blood vessels brought about due to the effect of the increased pressure of CO_2 in the blood.

observed: instead of the increase, a decrease of the blood supply of the brain takes place. The observed changes of the responses of the brain vessels under the interoceptive excitation during and after hypercapnia are due to the changes in the functional condition of the wall of the blood vessels brought about under the effect of the increased pressure of CO_2 in blood.

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* In Russian.

THE RELATIONSHIP BETWEEN THE BLOOD PRESSURE REFLEX ARISING IN THE PRESSORECEPTORS OF THE SMALL INTESTINE AND METABOLISM IN THE RECEPTOR AREA

O. P. Dobromyslova

From the Department of Normal Physiology (Prof. A. A. Zubkov, Chairman) of the Kishinev Medical Institute

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The interoceptors are intimately connected with the tissues of the organs in which they are located. One may suppose, therefore, that the metabolism in the tissues and consequently also in the interoceptors is one of the essential features which change the nature of the response from these interoceptors. Confirmation of the correctness of such a view is found in the work of V. N. Chernigovsky, V. A. Lebedeva and M. L. Belenky [1, 5], from which it is apparent that a change in metabolism in the chemoreceptors of the carotid sinus effected by various means (the action of carbon dioxide, cyanides, lack of oxygen, inhibition of glycolysis by means of sodium flouride and monoiodoacetic acid) leads either to a change in the strength of the reflex or to a distortion of the reflex response arising from these interoceptors.

In the present research we set before ourselves the task of studying the influence of changes in metabolism in the wall of the small intestine, and thus also in the pressoreceptors located in the intestinal wall, on the nature of the reflex response arising from these pressoreceptors.

A. A. Ukhtomsky [6] considered that excitation in nervous tissue, and consequently also in the receptors, consists in the harmony of two processes: the production of a peak of action current and a compensation reaction which restores readiness to produce a new peak of action current. The restoration of normal excitability, as was shown by A. A. Ukhtomsky and his co-workers, takes place as a result of the metabolism of this tissue, the essential links of which are the disintegration of adenosinetriphosphate, the disintegration of phosphocreatine, glycolysis and, according the contemporary data, respiratory oxidation as well. Inasmuch as glycolysis is one of the slowest parts of the compensation reaction, one may suppose that strengthening or weakening it can correspondingly strengthen or weaken the compensation reaction in the receptors, thereby changing the intensity of the afferent impulsation arising in them and consequently the intensity of the corresponding reflex response. The more intensive the compensation reaction, i. e., the more rapidly the tissue is prepared for the production of a new spark of excitation, the greater will be the amount of impulsation which a receptor can conduct in a unit of time; in other words, the lability of the receptor increases. When the compensation reaction is weakened, the opposite relationships will prevail.

We studied the influence which is exerted by a strengthening or weakening of glycolysis in the wall of the small intestine on the nature and magnitude of the reflex obtained during irritation of the pressoreceptors located in this intestinal wall. Our previous research [3, 4] showed in prolonged experiments on dogs with isolated intestinal loops that the reflex influence of one intestinal loop on the secretion and motor activity of another loop changes with a strengthening or weakening of glycolysis. If prior to irritation of the pressoreceptors substances are injected which some way or another intensify carbohydrate metabolism in the tissues of the intestinal wall, and at the same time in the pressoreceptors located in it (epinephrine, glucose, glucose with insulin), a strengthening of the reflex can be noted. Inhibition of glycolysis in the intestinal wall by means of monoiodoacetic

cid leads, on the contrary, to the prevalence of inhibitory influences from those pressoreceptors being stimulated.

In the experiments described below this problem was resolved by observing the reflex effects of the presso-ceptors of the small intestine not on its motor and secretory activity, but on blood pressure. The blood pressure eflex arising in the pressoreceptors of the small intestine was selected by us in view of its great clearness and tability.

EXPERIMENTAL METHODS

The experiments were performed on cats under urethane narcosis (1 g of urethane per kg of body weight njected intraperitoneally), which were injected against a background of ether narcosis.

Blood pressure was recorded in the carotid artery. The abdominal cavity was opened and a loop of the nall intestine withdrawn. Irritation of the pressoreceptors of the small intestine was accomplished with a rubber alloon (at 60-80 mm of mercury) which was introduced into the intestine or with a rubber collar located on the utside of the intestinal loop which also created a pressure against the outside of the loop amounting to 60- f mercury. Inasmuch as irritation of the pressoreceptors by pressure on the outside of the intestine produced a nore distinct reflex change in blood pressure than irritation of the pressoreceptors of the same portion by inflating ne balloon on the inside, we chose the first method of stimulation. In all of the experiments we employed a 0-15 minute irritation. In addition we did not apply continuous, but rather rhythmic stimulation: 10 constric-ions of the intestine during 10-15 minutes of irritation.

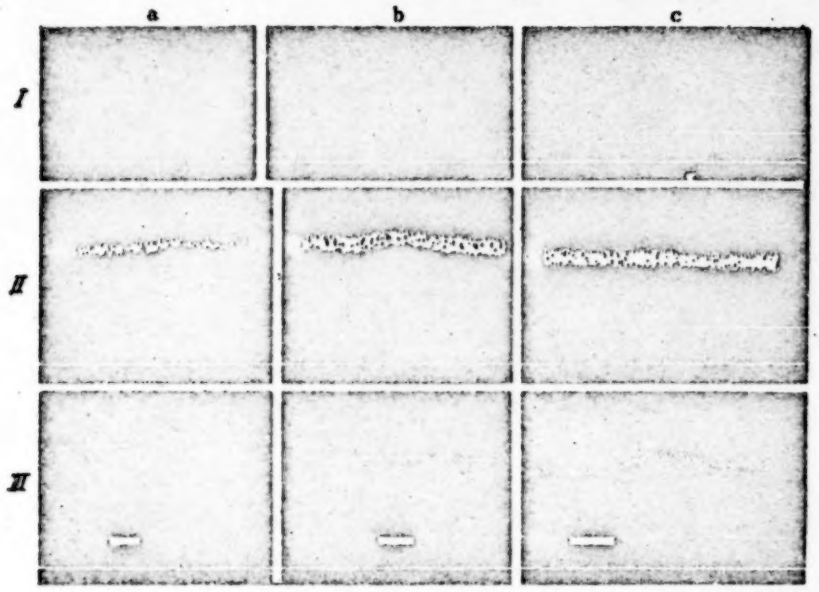

Fig. 1. The blood pressure reflex arising in the pressoreceptors of the small intestine before and after the influence of various substances on the receptor area. I. a) Normally; b) 5 minutes after injection of epinephrine; c) 15 minutes after injection of epinephrine; II. a) normally; b) following injection of glucose; c) following injection of insulin; III. a) normally; b) after injection of monoiodo-acetic acid; c) after the addition of lactic acid. From above down: blood pressure record, mark indicating the irritation, time scale (30 seconds).

EXPERIMENTAL RESULTS

Mechanical irritation of the pressoreceptors of the small intestine results in a short-term rise in blood pres-ure. Prior cocainization of the serosa of the small intestine leads to the disappearance of the reflex. The

reported elevation of blood pressure can be observed arising from any portion of the small intestine. It is more distinctly marked in male cats than in females: the reflex described was observed in 15 out of 16 male cats, while it was absent in two out of three females and was very weakly expressed in the one.

After the blood pressure reflex was obtained from one portion of the small intestine, through the needle of a syringe there was injected into the lumen of that portion a substance which exerts an influence on glycolysis: epinephrine, insulin, glucose, glucose with insulin, monoiodoacetic acid, sodium fluoride, dinitrophenol and lactic acid, i. e., agents acting on metabolism in different ways. We considered that, owing to the absorptive capacity of the mucosa of the small intestine, these substances should rapidly enter the intestinal wall. The active agent was also simultaneously applied to the serosa of the portion on which the experiment was being performed.

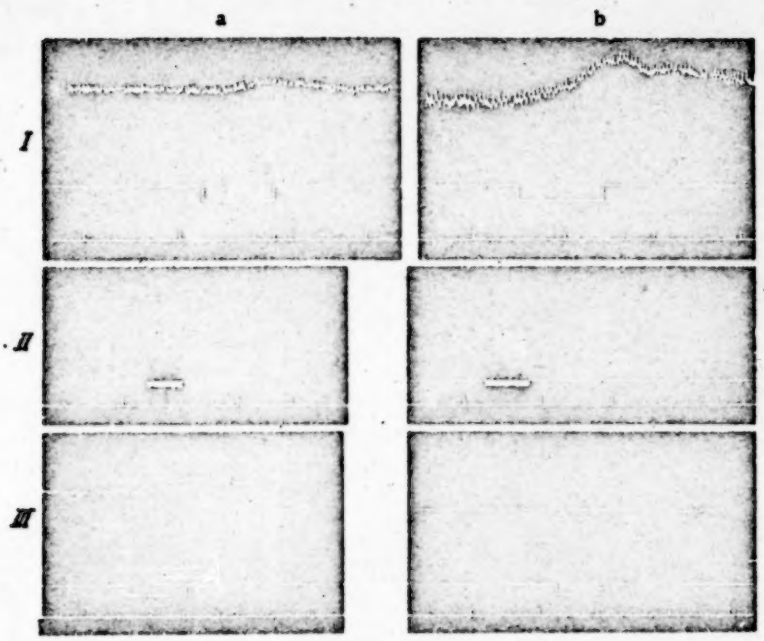

Fig. 2. The blood pressure reflex arising in the pressoreceptors of the small intestine before and after the influence of various substances on the receptor area.
I. a) Normally; b) after injection of glucose with insulin; II. a) normally; b) after injection of sodium fluoride; III. a) normally; b) after injection of 2,4-dinitrophenol. Designation of the curves is the same as in Fig. 1.

When the normal pressor reflex was obtained, 1 ml of solution of epinephrine in a dilution of 1:1000 was injected into the same portion. Irritation of the intestine following the injection of epinephrine resulted in a more distinct pressor reflex (Fig. 1,I). Intensification of the pressor reflex was occasionally observed for as long as 20 minutes after injection of epinephrine, but in the majority of the experiments it was maintained only for a period of 10-15 minutes. In those cases in which an original pressor reflex was absent, injection of epinephrine led to its appearance. Injection into the intestine of the same amount of epinephrine in dilutions of 1:10,000 and 1:100,000 did not influence the magnitude of the reflex. Epinephrine cannot produce its proper pressor reflex when injected into the hepatic portal system [2].

Glucose (a 5% solution) was injected in the amount of 3-4 ml, insulin in the amount of 1 ml. These two agents exerted the opposite effect on the reflex arising in the pressoreceptors, a fact which was especially noticeable when from two different portions of the intestine of the same animal initial blood pressure reflexes of the

same magnitude were obtained: if glucose was injected into one of these portions and insulin into the other, the reflex arising from the portion into which glucose had been injected became more distinct, while that arising from the portion which had been subjected to the action of insulin disappeared entirely (Fig. 1,II). The intensifying effect of glucose, however, was considerably less than that of epinephrine. Increasing the concentration of glucose above 5% did not lead to an intensification of the reflex.

If insulin was injected into the intestine together with glucose (1 ml of insulin+4 ml of 5% glucose), the magnitude of the blood pressure reflex increased considerably more than when glucose alone was injected. Therefore the effect produced by the joint injection of insulin and glucose cannot be regarded as the simple summation of the effects of insulin and glucose (Fig. 2,I).

Injection into the intestine of substances which inhibit glycolysis (monoiodoacetic acid, sodium fluoride) as well as dinitrophenol, which curtails respiratory phosphorylation, reduced the magnitude of the pressor reflex or led to its complete disappearance.

Monoiodoacetic acid was injected in the amount of 1-2 ml in dilutions of 1:1000 to 1:100,000. The most marked effect of monoiodoacetic acid took place when it was used in a dilution of 1:1000. Irritation of the pressoreceptors of the small intestine after injection of this agent led at the beginning to reduction, and then to the complete disappearance of the blood pressure reflex arising in these pressoreceptors (Fig. 1,III).

Sodium fluoride (2 ml of a 1% solution), unlike monoiodoacetic acid, did not result in the complete disappearance of the pressor reflex but only reduced it (Fig. 2,II). While mechanical irritation of the small intestine following injection of monoiodoacetic acid produced a weaker and weaker pressor reflex and finally its disappearance, mechanical irritation following injection of sodium fluoride resulted in a depression of the pressor reflex only in the first few minutes after this preparation was used. Subsequently the magnitude of the reflex was rapidly restored. Injection of another dose of sodium fluoride led once again to a considerable reduction in the magnitude of the pressor reflex with the same rapid (within 10-15 minutes) restoration.

2,4-dinitrophenol (4 ml of a 0.05% solution) had an effect similar to that of monoiodoacetic acid: irritation of the pressoreceptors of the intestine following injection initially produced a reduction of the pressor reflex and then its complete disappearance (Fig. 2,III). The disappearance of pressor reflexes was extremely persistent: after dinitrophenol was injected into several portions of the small intestine, reflex elevation of blood pressure could not be obtained from any one of these or neighboring portions for as long as 2 hours after the dinitriphenol was used.

We also tried the following variation of the experiment. After monoiodoacetic acid had led to the disappearance of the pressor reflex, into the same portion of the intestine we injected lactic acid (2 ml of a 0.5% solution), i. e., one of the end products of glycolysis. Irritation of the pressoreceptors of the small intestine after injection of lactic acid led to the restoration of the lost pressor reflex (Fig. 1,III).

Thus the change in the unconditioned interoceptive blood pressure reflex arising in the pressoreceptors of the small intestine depends first of all on the state of metabolism in the receptor area.

In the experiments cited above it was shown that injection into the intestine of substances which intensify carbohydrate metabolism by stimulating glycolysis in one way or another (epinephrine, glucose, glucose with insulin) produces a change in the blood pressure reflex arising in the pressoreceptors of the intestine, manifested by the augmentation of the reflex.

Injection into the intestine of monoiodoacetic acid and sodium fluoride, substances which inhibit glycolysis, of insulin without glucose (diverting the carbohydrate resources to the synthesis of glycogen) and of dinitrophenol, which curtails respiratory phosphorylation, results in the reduction or disappearance of the blood pressure reflex arising in the pressoreceptors of the corresponding portion of the intestine. Injection into the intestine following monoiodoacetic acid of an end product of glycolysis, lactic acid, restores the reflex arising from the corresponding receptor area, which had been lost after use of monoiodoacetic acid.

The results of our experiments agree with the results of our earlier research which included a prolonged experiment and allow one to conclude that in supplying energy for the work of the pressoreceptors of the intestinal wall an essential role is played by metabolic processes of restitution, in particular glycolysis and respiratory phosphorylation.

SUMMARY

Experiments based on recordings of the reflex response of the blood pressure in cats to mechanical stimulation of pressoreceptors in the wall of the small intestine show that different agents affecting tissue metabolism when introduced into the intestinal lumen and applied to its surface cause changes in the reactivity of the intestinal pressoreceptors. Solutions of epinephrine, glucose and glucose with insulin increase the pressor reflex from the intestinal pressoreceptors while solutions of monoiodoacetic acid and sodium fluoride (inhibition of glycolysis) as well as 2,4-dinitrophenol (inhibition of phosphorylation connected with respiration) decrease it to the point of total disappearance. Insulin acts in the same manner; lactic acid restores the reflex inhibited by monoiodoacetic acid.

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CHANGES IN LABILITY AND SPEED OF ACCOMMODATION OF SPINAL CORD CENTERS DURING INHIBITION AND GENERAL ANESTHESIA

A. D. Reva

From the Department of Human and Animal Physiology and Biochemistry (Lecturer P. E. Motsny, Chairman) of the Dnepropetrovsk State University Dedicated to the 300th Anniversary of the Reunion of the Ukraine with Russia

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In setting forth the theory of parabiosis, N. E. Vvedensky [1] emphasized that stimulation impulses functioning inhibitorily and alternative influences can replace each other in the emergence of the parabiotic state. From this point of view narcosis can in a wide sense be characterized as a kind of inhibition resulting from factors acting locally, while inhibition, produced, like narcosis, through physiological factors, is the result of nervous impulses. In both of these cases the inhibition represents stimulation confined to the site of its emergence. The common nature of the states of anesthesia and inhibition, according to N. E. Vvedensky, consists in the fact that in both of these states a sharp decrease in functional mobility is observed.

In experiments on spinal cats P. E. Motsny [2] detected a lowering of functional mobility and an increase in the speed of accommodation in the motor centers of the spinal cord during reciprocal inhibition. We have shown [3, 4] that during narcosis, together with the depression of reflex reactions, there is observed a lowering of functional mobility and an increase in the rate of accommodation in the motor centers of the spinal cord.

In the present research we set ourselves the goal of determining the nature of the functional changes which arise in the reflex centers of the spinal cord during reciprocal inhibition in the initial stages of general anesthesia and also of explaining whether or not the functional changes which arise are summational. The summation in the reflex centers of the spinal cord of the functional changes produced by inhibition and the changes caused by anesthesia would be one proof of the common nature of central inhibition and anesthesia.

EXPERIMENTAL METHODS

Experiments were performed on spinal cats and, in a few cases, on decerebrated cats. The narcotic agents used were: ether or chloroform as inhalation anesthetics; as intravenous or intraperitoneal anesthetics—Pentothal Nembutal and Hexenal. Recording of functional mobility and speed of accommodation in the reflex centers of the spinal cord was carried out by the myographic method [3, 4]. For the direct irritation of the motor centers of the spinal cord corresponding to the tibialis anterior and gastrocnemius muscles, the spinal cord was opened at the level of segments $\alpha_6 - S_1$ and a microelectrode (a silver wire 30 μ in diameter sealed in a glass capillary tube) was introduced from the superior surface. The position of the active tip of the microelectrode in the spinal cord was checked by histological sectioning in layers of that portion of the cord containing the electrode. Irritation of the centers was performed by means of single broken-current stimuli applied unipolarly (the anode was place on the spinal muscles). The changes in lability and speed of accommodation in the center of the spinal cord during inhibition and anesthesia were judged by the change in correlation of the height of contraction of the tibialis anterior or gastrocnemius muscle in response to the direct irritation of their centers with currents of different strength and duration. As an inhibiting nerve, for the tibialis anterior the popliteal nerve of the opposite paw was selected, and the deep peroneal nerve of the same paw was taken for the gastrocnemius. In the first

case the inhibitory volley preceded the positive one by 40-60 milliseconds, in the second by 5-10 milliseconds. The duration of the interval between irritations was determined with the use of a Hemholtz Pendulum.

In the beginning of the experiment (before anesthesia) the initial level of functional mobility and the speed of accommodation of the center under investigation were recorded. Moreover, in recording the speed of accommodation a steady ratio was obtained between the height of contraction of the muscle in response to direct irritation of the center with a rectangular current and the height of its contractions on irritation of the same center with an exponentially increasing current (H_c:H_z). In recording the background of functional mobility a steady ratio was obtained between the height of the contractions of a muscle in response to the direct irritation of its center with a current of short duration and the height of contractions in response to a current of long duration (H_c:H_d). Then the second set of these contractions of the muscle being tested were recorded, but this time against a background of a preceding volley from the inhibiting nerve. After a steady background was recorded in the normal condition and during inhibition, the animal was anesthetized and the recording of the muscular contractions was carried on simultaneously in the indicated sequence. The recording of the contractions of a muscle was accomplished according to the extent to which the animal had come out of the state of anesthesia.

EXPERIMENTAL RESULTS

The experiments showed that inhibitory volleys during light anesthesia produce in the motor centers of the spinal cord a greater than normal reduction in functional mobility.

The graph of one of the experiments, which is presented in Fig. 1, shows that an inhibitory volley induced by irritation of the contralateral popliteal nerve in the normal condition (before anesthesia) resulted in a 26% reduction in functional mobility in the motor center of the tibialis anterior. Sodium pentothal (1 ml of a 2% solution) injected intravenously into a spinal cat weighing 2.1 kg reduced functional mobility by 28%, and these same inhibitory volleys, but against a background of the animal's anesthesia, resulted in a 94% reduction in the functional mobility of the same center, i. e., more than 3 times the magnitude of the reduction in functional mobility by inhibitory volleys in the normal state. Thus during contralateral inhibition of spinal cord reflexes against a background of mild anesthesia a greater reduction in functional mobility is observed in the motor center of the tibialis anterior than during inhibition or anesthesia alone.

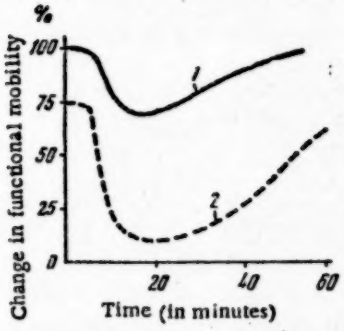

Fig. 1. Change in functional mobility in a motor center of the spinal cord of a cat (in % of the normal value) during anesthesia (1) and inhibition against a background of anesthesia (2). Test muscle: the tibialis anterior. The moment of injection of sodium pentothal is designated with an arrow.*

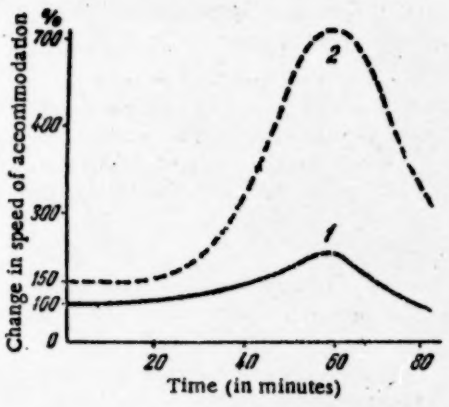

Fig. 2. Change in speed of accommodation in a motor center of the spinal cord of a cat (in % of the normal value) during anesthesia (1) and inhibition against a background of anesthesia (2). Test muscle: the tibialis anterior. The moment of injection of nembutal is designated with an arrow.*

Figure 2 contains a graph of another experiment, which illustrates the change in the speed of accommodation

*Figures do not show arrow -- Publisher.

in the motor center of the tibialis anterior during anesthesia and contralateral inhibition against a background of anesthesia. The speed of accommodation of the motor center in the normal state was taken as 100%. The graph shows that during contralateral inhibition the speed of accommodation in a motor center of the spinal cord increases up to 152%. Intraperitoneal injection of 2 ml of a 2.5% solution of sodium nembutal into a spinal cat weighing 2.3 kg caused an increase in the speed of accommodation of the same center (after 50 minutes) of up to a maximum of 214%, while the same inhibitory volleys entering the motor center against a background of anesthesia caused an increase of up to 720% in the speed of accommodation of the same center, i. e., almost 4 times greater in comparison with its increase during inhibition in the normal state.

The data adduced show that nervous impulses entering the motor center of the tibialis anterior muscle from the contralateral popliteal nerve produce in it during anesthesia a considerably greater increase in speed of accommodation in comparison with the normal speed.

Analogous changes in functional mobility and speed of accommodation were also detected by us in the motor center of the gastrocnemius muscle during ipsilateral inhibition against a background of light anesthesia.

The observations showed that both contralateral and ipsilateral inhibition promotes a greater decrease in functional mobility and a greater increase in speed of accommodation in the motor centers of the spinal cord among animals which are in the state of anesthesia, regardless of by what method and by which anesthetics this anesthesia was produced. In our experiments the phenomena described were observed during anesthesia caused by inhalation of ether or chloroform as well as by intravenous injection of Pentothal and Hexenal and intraperitoneal injection of Nembutal. The intensity and duration of the changes which arise, however, depend both on the dose and nature of the anesthetic and on the method of introducing it into the organism.

A comparison of the functional changes which are characteristic of parabiosis with the functional changes observed in the motor center of the spinal cord during inhibition against a background of anesthesia enables one to characterize the latter as a typical state of Vvedensky's parabiosis.

Deepening by inhibitory volleys the parabiosis of the motor centers of the spinal cord which was produced by the anesthetics attests to the summation of the functional changes caused by both of these factors, a fact which is in agreement with the teaching of N. E. Vvedensky on the common intrinsic nature of inhibition and anesthesia.

As regards changes in functional mobility and speed of accommodation in the premotor elements of the spinal cord, in the given set of experiments, when two factors – inhibition and anesthetic – act simultaneously on the centers of the spinal cord, there is not observed in them the contrasting changes which were noted earlier by us [3, 4] in the indicated functional parameters in comparison with those of the motor centers. This is apparently explained by the fact that the parabiosis of the motor centers of the spinal cord, caused by the anesthetics and strengthened by inhibitory impulses, also encompasses in the given case the region of the premotor elements, as a result of which changes in lability and speed of accommodation are detected in them which, in their direction, are the same as those in the motor centers.

SUMMARY

Changes in the functional properties of the spinal cord during reciprocal inhibition and anesthesia were studied in spinal cats by means of direct stimulation of the spinal centers. It was noted that, during anesthesia, ipsilateral and contralateral inhibition of spinal reflexes caused a greater reduction of functional mobility and an increased rate of accommodation in the spinal motor centers than in the case of anesthesia or inhibition alone. The summation in the motor centers of functional changes caused by anesthesia and changes originated by inhibition, conforms with N. E. Vvedensky's theory on the common intrinsic nature of inhibition and anesthesia.

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ADDITIONAL DATA ON THE CHANGES IN THE EFFICIENCY OF MUSCLES UNDER THE INFLUENCE OF THE ACTIVITY OF OTHER GROUPS OF MUSCLES

V. V. Rozenblat

From the Department of Normal Physiology (Prof. N. K. Vereshchagin, Chairman) of the State Medical Institute and from the City Medical and Physical Culture Dispensary (M. B. Kazakov, Head Physician), Sverdlovsk

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As we showed earlier [9], Sechenov's phenomenon (an increase in the efficiency of muscles under the influence of the prior activity of other groups of muscles) is distinctly expressed on a mercury dynamometer during static tension of the wrist, which was the object of our study. Moreover, in full agreement with the data of M. E. Marshak [4], S. P. Narikashvili and Sh. A. Chakhnashvili [6] as well as the recent data of S. I. Krapivintseva [1, 2], it was shown that the greatest effect is produced by work of other muscles which is characterized by a known moderate intensity and a moderate degree of fatigue; light or, on the other hand, excessively fatiguing work is scarcely effective. These facts support the idea that, in the intercentral influences which determine the effect of active rest, a leading role is played by the induction relationships which prevail, according to I. P. Pavlov [7], under just such conditions, i. e., not during weak, nor during excessively strong, but during moderately strong excitation of the cortical cells. The centers of the muscles being studied as other muscles are working show up in virtue of the simultaneous negative induction by the inhibited ones. If they were fatigued, a protective process of inhibition restores their strength more rapidly. The competence of the centers which were not working and were not fatigued also increases, but to a lesser extent.

The final question – that of the changes in the efficiency of the muscles which were not fatigued and were not working, under the influence of the activity of other groups of muscles – became the theme of our further research.

In the first series of examinations (76 observations of three subjects), performed under our direction in 1952 by a student, G. A. Dedyurkina, a study was made of the influence on the static endurance of the wrist (the duration of static tension on a mercury dynamometer under a load amounting to half the strength of the muscles being tested) of simultaneous, less intensive work by the other hand – static tension under a load amounting to one third of the strength. The given form of work by the other hand, used earlier by us as one of the types of active rest, afforded the greatest effect, i. e., with it, if it was used during rest, the induction relationships were most clearly evident. It was therefore of interest to explain what influence will be exerted by its use simultaneously with the work of the hand being examined.

Both in the fundamental and in the control observations, after the work of the hand being examined ceased, the subject was given 1½ minutes of rest, and then the static tension was repeated with the same load (but this time without including the other hand). This permitted one to judge indirectly the degree of fatigue in the first period of work, because the greater it is, the smaller will be the duration, other conditions being equal, of the repeated work after a short interval of time.

In handling the data obtained, as in our preceding work, we made use of the "individual relative values": for each hand of the individual subjects the average data of the control experiments were taken as 100%, and all

the results of the experiments with a given hand were expressed in percentages of this value. This provided a proportional handling of the data according to statistical variation. The averages of the relative individual values are cited in the third column of Table 1.

The data obtained (Table 1) show that worked performed simultaneously by the other hand increases the capacity for work of the muscles under investigation. This agrees with the data of E. A. Mukhamedova [5] and some other authors.

The increase in capacity for work is caused in our opinion by a mechanism of dominance. Proprioceptive impulses from the moderately taut muscles of the other hand are "attracted" to the dominant focus and stimulate it.

What is interesting in our data is that as an "accompanying" load that form of work was performed by the other hand which, during its performance before and after the work of the symmetrical muscles, produced inhibition in their centers in virtue of marked negative induction. Performed simultaneously with the work of the centers under investigation, it exerted the opposite influence on them: if the induction relationships were preserved in this instance, we would have obtained, not stimulation, but depression of the capacity for work (G. V. Popov [8] obtained just such a result during a corresponding set of experiments).

TABLE 1

Subjects	Hand	Simultaneous tension other hand (A)		Ordinary conditions (B)		A/B, in %	Duration of second work period (after 1.5 min. rest) in sec.	
		no. of measurements	average endurance in sec.	no. of measurements	avg. endurance (sec.)		A	B
D.	Right	7	67.1	7	63.1	106.3	45.0	50.0
	Left	7	66.7	5	59.8	110.0	45.3	47.0
Zh.	Right	7	55.4	7	55.4	100.2	39.4	40.8
	Left	7	51.8	6	43.5	119.2	37.5	37.7
U.	Right	6	50.2	6	45.8	109.5	40.0	32.5
	Left	6	54.2	5	45.0	123.1	34.2	35.0
Average			57.7		52.5	111.1 ±2.4	40.4	40.8

Notation. The probability integral (the reliability of the difference between the average measurements during the simultaneous work of the other hand and under ordinary conditions) amounts to 0.9996.

A comparison of the data of the 4th and 5th columns of Table 1 shows that the repeated (after $1^1/_2$ minutes) work of the hand under investigation was of the same duration in the fundamental and control experiments. Consequently the simultaneous work of the other hand postpones the development of exhaustion, not through disturbing the functions of the protective protective inhibition which later asserts itself (this would lead to a more marked exhaustion and to shortening the repeated work), but as a result of a real increase in competence under the influence of the stimulation of the dominant focus.

The data obtained show that a more careful look is required at the "superfluous" movements in athletics. At times supplementary movement (that, for example of the free hand of a thrower etc.) may even heighten the effect of the work.

In the second series of our examinations (70 observations of six subjects) a study was made of the influence of prior static tension of the other hand on the strength of the wrist which was not working (earlier in all our other examinations we studied the influence of this factor on another characteristic – endurance).

The strength of the hand under investigation (the right hand among 2 subjects and the left hand among 4) was measured with the use of a mercury dynamometer before and after static tension of the other hand continued

to the limit, i. e., until marked exhaustion, at which point continuation of the work was only possible with a reduction of the load. Control observations showed that with the time intervals selected repeated measurements by themselves do not provide substantial shifts in strength.

The data obtained (Table 2) show that the strength of the hand which was not working, measured immediately at the cessation of the work performed by the other hand, was in all 6 of the subjects greater than the initial level. Moreover, in two of the subjects the increase in strenght continued for yet a period of 30 seconds, and only during subsequent measurement – 2 minutes later – was there noted a reduction in strength and a return to the initial value. Among four subjects the maximal increase in strength was noted immediately after the cessation of the work of the other hand.

The fact of an increase in the strength of one hand under the influence of the work of the other corresponds to the data of A. N. Krestovnikov [3]. The special feature of our results was the fact that among a part of the subjects the increase in strength continued for a known period after the cessation of the work of the other hand.

This evidently indicates the duplex mechanism of that temporary increase in excitability of the corresponding cortical cells which is the basis of the observed short-term increase in the strength of the wrist.

If during the work of the other muscles the centers under investigation are in state of induced inhibition, then at the cessation of the work of the other hand the state of inhibition, in virtue of successive positive induction, gives place to a state of increased excitability. The given mechanism emerges distinctly in those two subjects (Ch. and V., see Table 2) in which was noted a gradual increase in strength. The induction relationships which prevailed during the work of the symmetrical muscles may, toward the end of their work, (when the development of fatigue and the corresponding increase in volitional tension considerably augments the excitation of the working centers) give place to irradiation of excitation (the neighboring muscles do not accidentally contract involuntarily during fatigue). This increases the excitability of the symmetrical centers, and this means the strength of the muscles as well. The given mechanism stands out distinctly among those four subjects in which the maximal increase in strength was noted immediately after the cessation of the work of the other hand (see Table 2.

TABLE 2

Subjects	No. of measurements	Average strength value (cm of mercury)			
		before work of other hand	right after work of other hand	after 30 sec.	after 2 mins.
M.	13	116.7	123.2	120.7	120.2
Ch.	7	114.0	115.7	116.8	114.0
Kp.	14	113.9	116.0	112.8	114.0
K.	12	118.5	121.2	118.6	116.5
Sh.	12	115.8	119.0	116.9	116.6
V.	12	117.8	118.8	119.1	118.9

Thus the facts obtained in comparison with the results of preceding research enable one to approach a precise statement of certain mechanisms of these intercentral relationships which produce a change in the working capacity of muscles under the influence of the work of other groups of muscles.

One and the same form of work by definite muscles (i. e., one and the same degree of proprioceptive impulsation into the corresponding centers) may exert a different influence on the other centers depending on their state: during relative rest it produces and strengthens inhibition in them (according to the principle of induction); during excitation it increases their activity (according to A. A. Ukhtomsky's principle of dominance).

The increase in the strength of certain muscles after the work of others is linked both with irradiation of excitation and with successive positive induction (following the induced inhibition during the work of the other muscles).

SUMMARY

A mercury dynamometer was used to study changes in the working capacity of muscles of the hand as influenced by the work of symmetrical muscles of the other hand. The first series (76 observations of 3 subjects) showed that endurance toward static tension (measured under a load equaling one half of the strength of the tested muscles) increases during simultaneous moderate tension in the other hand equaling one third of its strength. The second series (70 observations of 6 subjects) showed that the grasping power of the hand grows when static tension in the other hand is continued to the point of fatigue; sometimes this growth went on for several seconds after work had ceased. The central nervous mechanisms of the observed changes were analyzed.

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TOTAL SIMULTANEOUS EXCLUSION OF VISUAL, AUDITORY, OLFACTORY AND VESTIBULAR RECEPTORS IN ADULT ANIMALS

B. N. Klosovsky and E. N. Kosmarskaya

From the Department of Brain Development (Chairman – B. N. Klosovsky, Associate Member of the Academy of Medical Sciences of the USSR) of the Institute of Pediatrics of the Academy of Medical Sciences of the USSR (Director – O. D. Sokolova-Ponomareva, Associate Member of the Academy of Medical Sciences of the USSR)

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Our experiments have shown that depriving the nervous cells of the cerebral cortex of puppies three weeks to three months old of the impulses from the visual, auditory, olfactory and vestibular receptors is not accompanied by "passive" sleep. A few days after simultaneous total exclusion of the receptors mentioned the behavior of the experimental puppies does not differ from the behavior of normal animals of the same age [3].

At the same time, according to the paper by V. S. Galkin [1], in his experiments the simultaneous cutting of optic nerves (or enucleation of the eyes), destroying the chochleae and cutting the fila olfactoria resulted among adult dogs in a deep, almost comatose sleep. After the three receptors were destroyed, the dogs woke up independently only to urinate and defecate, external stimuli succeeded in waking them with difficulty, and even then they remained awake only a few seconds. As a result of extensive inhibition in the cerebral cortex, stimuli from the gastrointestinal tract did not succeed in dogs' waking due to the sensation of hunger. Therefore the dogs did not eat independently. In spite of artificial feeding the dogs lost weight and even died from emaciation.

Hence, in spite of the fact that in our experiments the cerebral cortex was deprived of a considerably greater quantity of stimuli from the external environment than in the experiments of V. S. Galkin, uninterrupted sleep was not observed. The disparity between the data which we obtained and those of V. S. Galkin can be explained in the following way. First of all it must be pointed out that the method which we employed to exclude the remote receptors differed from the method used in the experiments of V. S. Galkin by a more complete cutting of the optic nerves and, especially, in the destruction of the peripheral olfactory receptors in the nasal cavity. The extracranial exclusion of receptors, moreover, prevented trauma and infection of the brain material [2]. Aside from a somewhat different method of operation, the result of the experiments may be linked with the age of the animals.

As has already been stated, V. S. Galkin destroyed the receptors of adult dogs, i. e., after the animals' brains were already fully mature morphologically and the definitive sum of the links between the organisms and the internal and external environment was established. We destroyed the peripheral divisions of the optic, auditory, olfactory and vestibular analysors of young animals at that time at which intensive processes of development were going on in their brains and links with the internal and external environment were being established. A study of the dogs' brains 4-7 months after the operation showed that, in the absence of a large number of stimuli from the external environment, on the whole the brain remains underdeveloped. Especially underdeveloped were the central termini of those brain analysors, the peripheral divisions of which were excluded by the operation [3].

Setting before ourselves the task of establishing the consequences of excluding the four groups of remote receptors in adult animals, we performed the operation on one adult dog and 30 adult cats. As in the experiments on the puppies, a few days after the operation the thoroughness of the exclusion of the receptors was checked in all of the animals. In not a single one of them was there detected excitability of the visual, auditory, olfactory and vestibular receptors. In this series of experiments a continuous record was also kept of the behavior of the

animals during the working day (from 9 AM to 4 PM), as well as an actographic recording of their movements.

The table below, in which the results of the observations of the dog subjected to the operation are compiled for a period of one month following the operation, provides proof of the fact that depriving the brain of an adult dog of a large amount of the stimuli from the external environment does not involve the animal's uninterrupted sleep. On the contrary, after the exclusion of vision, hearing, smell and the vestibular apparatus of the dog there was noted an alternation of periods of varying duration of being asleep and awake. In addition the total duration of daytime sleep fluctuated on different days within wide limits and did not differ from the sleep of normal adult dogs living under the same conditions.

The animals woke up independently in every instance, and their waking was not always accompanied by urination or defecation. At the same time the dog could easily be awakened by touching, by mild jarring of the walls of the enclosure near which it was sleeping, etc. In this respect our data are in complete agreement with the observations of N. F. Popov [4].

During the time it was awake, the duration of which in the course of a day could exceed 5 hours, the dog walked a lot, stood on its hind paws, climbed out of the enclosure over the low walls, etc. When walking in a room with a large amount of furniture, the dog stumbled over all the objects which it encountered. It by-passed some of them and crawled under others; finding itself by accident between things or in a corner, the dog always left the spot by moving backwards. On the first day after the operation it was observed that the animal had lost the feeling for the position of its body in space. In the enclosure the dog lay quietly, leaning against its wall; it did not stand up on its paws. If we attempted to place it in a free area of the enclosure where it did not touch anything, the dog jumped high in the air with its paws placed wide apart and fell on the floor, landing hard with its whole body. On the floor it threw out its paws wide and "stretched" with them until it encountered some object. After that the dog quieted down. If the object was moved away, the jump and all the phenomena following it were repeated again. All of this was accompanied by pronounced panting by the animal, which lay with its mouth open and its tongue protruding.

On the 2nd day after the operation the experimental dog was already standing on half-twisted, widely separated paws, but it did not walk. The phenomena which were observed on the first day after the operation disappeared: the dog was able to stand without touching anything, but in so doing it breathed heavily and often. The animal's excitation and panting were also noted on the 3rd day after the operation, when the dog began to walk about in the enclosure. On this day and on the following days disturbances of movement or of musculature tonus were not noted.

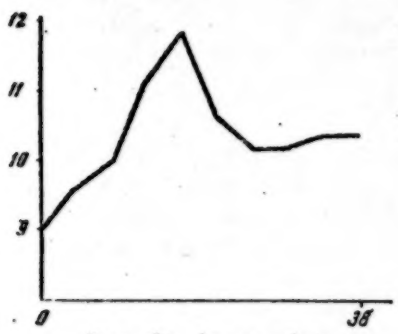

Fig. 1. The change in weight of the dog after total simultaneous exclusion of the four groups of remote receptors.

The experimental dog was already eating independently the day after the operation. Initially the correct slope of its head into the feeding trough was hindered by its strong sweeping movements from side to side. Toward the end of the day, however, the dog was taking meat from the trough just as it had done prior to the operation. On the second day after the operation the animal found the trough in the enclosure by itself and ate the food which had been left overnight. After eating the dog licked the pan frequently. While the dog ate, there was at times observed an agressive reaction toward the puppies which approached its feeding trough. The dog's taste was fully preserved after the operation, since it always selected the pieces of meat from the trough, and only after that did it eat the bread; it gnawed bones willingly, always holding them down with its paw. As can be seen from Fig. 1, after the operation not only did it not lose weight, but even increased it considerably.

The Duration of the Dog's Periods of Being Asleep and Awake

No. of the observation	Date	Duration of the periods of being asleep and awake in hours and minutes																Total duration in hours and minutes	
		awake	asleep	awake	asleep	awake	asleep	awake	asleep	awake	asleep	awake	asleep	awake	asleep	awake	asleep	awake	asleep
1	27.1	4.10	0.17	0 21	1.22	0.08	0.17	0.18	0.27	0.06	0.07	0.02	0.02	0.05	0.18	0.03	0.037	0.04		3.27	5.37
2	30/1		0.18	0.46	0.34	0.12	0.15	0.35	0.05	0.17	0.25	1.06	1.00							2.37	2.56
3	2/11	0.42	0.11	0.42	1.20	0.25	0.45	0.22	0.03	0.05	1.05									3.24	2.16
4	6/11		1.42	0.05	0.55	0.11	0.04	0.40	0.07	0.03	0.45	0.10	1.10							4.43	1.03
5	8/11	0.42	0.57	0.02	0.13	0.08	0.31	0.14	0.50	0.05	0.03	0.19	0.10	0.20	0.11	0.02	1.15			4.09	1.52
6	9/11	0.12	0.03	1.06	0.11	0.03	0.07	0.03	0.16	0.02	0.42	0.04	0.15	0.55	0.02	0.08	0.42	0.43	0.20	2.39	3.14
7	13.11	0.58	1.44	0.16	1.04	0.30	0.37	1.03	0.34	0.38	0.17									3.25	2.47
8	16/11	0.29	0.44	0.04	0.37	0.08	1.07	1.04	0.45	0.30	1.10									3.19	2.23
9	20/11	1.22	0.48	3.52																0.48	5.14
10	25/11	1.28	0.30	0.30	0.25	0.28	0.07	0.25	0.45	0.30	1.10									2.57	3.21

The behavior of the adult cats after total simultaneous exclusion of their vision, hearing, smell and vestibular apparatus was the complete opposite of the behavior of the adult dog which underwent the same operation.

In contrast to the dog, the adult cats, deprived of stimuli from the receptors mentioned, spent all the time in sleep. According to the data of Szymansky (1920) as well as our own data, domestic cats belong to a group of polyphase animals among which night sleep predominates over that of the daytime. While normal cats are awake for most of the day and sleep only for short intervals of time, the cats with remote receptors excluded slept the whole day (Fig. 2,a). The animals deprived of vision, hearing, smell and vestibular apparatus woke up only to urinate or defecate. Their sleep was extremely deep and was with difficulty disturbed by touching or shaking the walls of the cage, etc. At the same time the animals, while asleep, performed a certain number of movements (Fig. 2,b). The time awake was of extemely short duration and did not exceed 1-2 minutes.

In contrast to the full-grown dog, the adult cats for 5-7 days after exclusion of the four groups of remote receptors did not try to get up on their paws, but rather crept from place to place. Subsequently they stood on half-twisted paws, especially while eating, and even moved about over a very small distance. Some of the animals walked on fully straightened paws, but very few. A change in the animals' positions was observed only before or after feeding if the animals were spurred on by pushing. For the first 3 days after the operation the cats slept in cages, of necessity leaning against one of the walls. In a free area they began to crawl until they touched some object, beside which they rapidly fell asleep. Among a number of the adult cats the loss of the feeling for the position of the body in space was expressed to a considerably marked degree and was accompanied by the same phenomena which were observed in the adult dog the first day after the operation. The animals' jumping into the air, however, was accompanied by a marked agressive reaction: The animals howled loudly, bared their claws, hissed, etc. Subsequently the cats did not reveal an inclination to touch some object and could sleep peacefully in a free space.

In the majority of the adult cats upon which we operated, disturbed swallowing was noted during the first 1-3 days after the operation. Subsequently the swallowing reflex was restored, but with the exception of a single cat the animals did not eat independently and it was necessary to feed them artificially. Therefore the cat which began to eat independently a week after the operation kept its weight on the preoperative level for the entire 69 days of its life after exclusion of the receptors (Fig. 3,a). Among the other animals, in spite of the force applied, we were

not successful in keeping their weight on the preoperative level (Fig. 3,b,c). The cats never woke up by themselves in order to eat and did not look for the feeding troughs, even in those instances in which they had not been fed for 2 days. Among many of the animals for 12-20 days after the operation an agressiveness was observed, detected especially during feeding. It was characteristic that, during a strong irritation which was unpleasant for them, the adult cats which were deprived of the most important aspects of reception never localized the irritation. In response to strong irritation, in the region of the sacrum for example, a cat would hiss furiously, show its teeth, bare its claws, beat its tail, etc., but it would not turn its head to the side of the irritation, or try to catch the hand of the person irritating it, or even make an attempt to crawl away from the site of the irritation. One of the characteristic features of the adult cats following the exclusion of their vision, hearing, smell and vestibular apparatus was the loss of a feeling for cleanliness, which lasted, as a rule, for 2-3 weeks. During this time the cats did not "wash themselves", did not lick their coats and did not crawl away from a wet place.

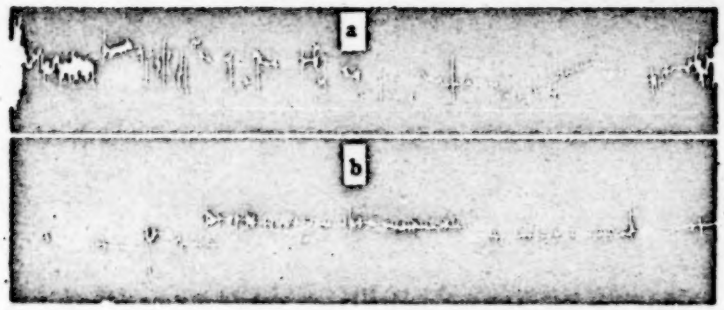

Fig. 2. Actograms of the motor activity of adult cats in the course of a day. a) Normal cats; b) experimental cats 1 month after exclusion of the four groups of remote receptors.

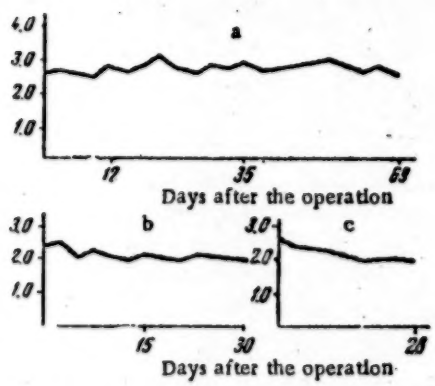

Fig. 3. The change in weight of adult cats after total simultaneous exclusion of the four groups of remote receptors. 1) Days after the operation; a) cats which lived 69 days after the operation; b) cats which lived one month; c) cats which lived 28 days.

Thus the experiments showed that a period of uninterrupted sleep is not observed as a result of simultaneous total exclusion of visual, auditory, olfactory and vestibular receptors in adult dogs, just as it is not among puppies three weeks to three months old. Among these animals sleep alternates with periods of being awake. Waking occurs independently or can easily be produced by contact; the periods of being awake are active. On the other hand among cats deprived of the same receptors a shut-down of deep sleep ensues. Independent waking occurs only in connection with urination and defecation and lasts for not more than 1-2 minutes.

The data obtained by us require further examination in order to shed light on the causes of the different behavior of the adult dogs and cats deprived of the four groups of remote receptors.

SUMMARY

The visual, auditory, olfactory and vestibular receptors of one adult dog and 30 adult cats were excluded on both sides during the same operation by a procedure elaborated earlier by the same authors. The paper describes the dog's behavior and data on the duration of sleep and wakefulness observed for 7 months after operation. It was demonstrated that exclusion of the receptors mentioned in adult dogs is not followed by uninterrupted sleep. Sleep and wakefulness succeeded one another and the duration of daytime sleep did not differ from normal. Awakening occurred spontaneously or was easily caused by touch.

Cats, on the contrary, deprived of the same receptors, developed profound sleep inhibition. Spontaneous awakening occurred only for urination and defecation and did not last longer than 1-2 minutes. The behavior of the cats after the operation is described.

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ON THE QUESTION OF THE FUNCTIONAL RESPONSE OF PAIRED SALIVARY GLANDS WHEN A PORTION OF THE ORAL MUCOSA IS ANESTHETIZED

A. P. Savinskaya

From the Laboratory of Experimental Pathology (Chief – Prof. S. I. Lebedinskaya)
Division of General Pathology (Chief – Academician A. D. Speransky) of the
Institute of Normal and Pathological Physiology (Director – Prof. V. N. Chernigovsky)

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When studying the inter-linked activities of paired parotid glands, we demonstrated that salivary secretion is not the same in each gland when unconditioned and conditioned stimulators were employed [2].

At the present time, it has been well established by the work of I. P. Pavlov and his school that the functional state of the corresponding nerve centers largely determines the intensity of salivary gland activity. From this it follows that, for the causes leading to a difference in the type and amount of secretion, we must examine the mechanism of the differences in the functional state of the corresponding centers in the right and left cerebral hemispheres.

To confirm this supposition, we attempted to set up an experiment which would permit some judgment as to the reasons for the unequal responses elicited from the paired glands.

It is accepted as a fact that the degree of excitation of the nerve centers is largely determined by the intensity of the afferent stimulation of the corresponding receptor zones. By altering the intensity of the impulses entering the central nervous system from the periphery, by increasing or decreasing their number, we can correspondingly increase or decrease the degree of irritability of the centers associated with them. Each parotid gland has been shown by the studies of K. S. Abuladze [1] to be stimulated by impulses reaching the center principally from the ipsilateral half of the oral mucosa.

We set up experiments having the aim of observing one-sided partial anesthesia of the oral mucosa and the effects produced by it upon the salivary secretion of paired parotid glands. Such experiments are able to demonstrate the significance of the usual stimuli of the oral mucosa and the response they evoke in the nerve centers and, hence, determine the quantity of salivary secretion under such conditions.

We are aware of only one such study having been made on the changes produced in unconditioned reflexes when salivary secretion of paired parotid glands was studied [4]. Analogous studies on salivary secretion employing conditioned reflexes, we have not uncovered.

EXPERIMENTAL METHODS

The experiments were conducted on dogs Red (strong, well-balanced type), Elsa (strong, unstable type) and Wolf (feeble type). These general characteristics of the animals were determined by three-year long studies of their response to conditioned reflexes and with the aid of special tests (experiments with caffeine, day-long fast, prolongation of the differentiation, "inaccuracy").

Up to the moment of conducting experiments with the anesthesia of a portion of the oral mucosa, definite

conditioned responses to bell ringing were established, metronome 120 strokes per minute, blinker light and one differentiation to metronome 120 – metronome 60 strokes per minute.

The anesthetizing substance used was Dicaine.

In the first series of experiments upon dogs we inserted inside the left cheek a tampon moistened with 5% Dicaine solution. The muzzle was held together firmly by means of the hand so that the dog could not roll the tampon to the other side. The cheek holding the tampon was massaged lightly. Then the tampon was removed. Beginning after 5-7 minutes following this procedure on the dog, the salivary secretion of the paired parotid glands was observed using the established conditioned reflexes. The Dicaine was applied outside the chamber. The depth and duration of the anesthesia was verified by using the solutions first on the experimenter. It would begin within 5 minutes of application and last 25-30 minutes.

At the moment of applying the tampon, all the dogs were observed to have salivary secretion from the left parotid gland.

EXPERIMENTAL RESULTS

In dogs of the strong, stable type (Red), commencing within 10 minutes after the Dicaine application, there was observed a gradual weakening of the conditioned reflex response on the anesthetized side until zero level was reached, then, by the time 30 minutes had passed, as the action of the Dicaine would disappear, there would be seen a complete restoration of the conditioned reflex response. The dog acted quite normally within the chamber, eating well.

The conditioned salivary secretion from the opposite side was not altered materially (Fig. 1).

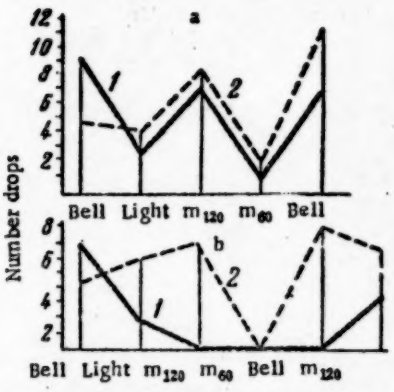

Fig. 1. Conditioned reflex salivary secretion in dog Red.
a) Normal; b) after anesthetizing left cheek with 5% Dicaine; 1) conditioned reflex salivary secretion from left parotid gland; 2) same from right.

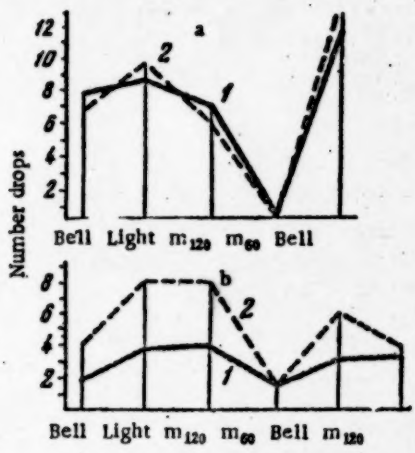

Fig. 2. Conditioned reflex salivary secretion in dog Elsa.
a) Normal; b) after anesthetizing left cheek with 5% Dicaine; 1) conditioned reflex salivary secretion from left parotid gland; 2) same from right.

In a dog of strong, unstable type (Elsa) conditioned reflex salivary secretion also diminished on the anesthetized side, beginning within 5 minutes of the application to the mucosa and remained at low levels throughout the duration of the experiment (30 min.) (Fig. 2). The behavior of the dog within the chamber was normal, food being well-consumed.

In a dog of the feeble type (Wolf) the salivary secretion of the conditioned reflexes was lowered from both glands, indeed, being greater on the side opposite the anesthesia. This was especially striking at the greatest depth of the anesthesia (15 min. after applying Dicaine). Only toward the end of the experiment (30th minute)

id the response to the positive stimulator m_{120} become equalized (Fig. 3). The behavior of the dog within the hamber was markedly altered. With the application of the first positive condioning stimuli, the dog Wolf had he usual reaction of movement: the appearance of the food would cause Wolf to throw himself on it, but, having onsumed only a portion of it, he would move away. After that, Wolf would eat only in the presence of the experimenter, first with hand re-enforcement, then, from the feed-box. He would take food independently only oward the end of the experiment.

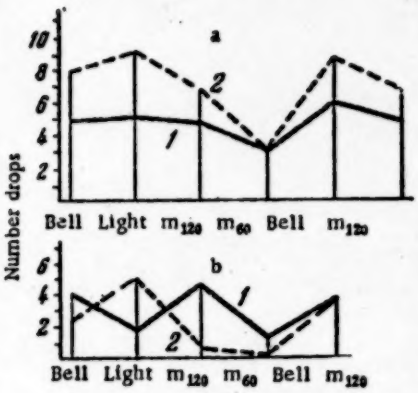

Fig. 3. Conditioned reflex salivary secretion in dog Wolf.
a) Normal; b) after anesthetizing left cheek with 5% Dicaine; 1) conditioned reflex salivary secretion from left parotid gland; 2) same from right.

In this fashion, it can be seen that anesthesia of a portion of the oral mucosa by use of 5% Dicaine in dogs of the strong type depresses conditioned reflex salivary secretion only on the anesthetized side, not altering the animal's reaction to food, while in a dog of the feeble type, reflex conditioned salivary secretion is altered in both glands, in addition the response to food being also changed.

In a second series of experiments, 10% Dicaine solution was used to produce the anesthesia.

The method used was identical with the first series.

In the experiments of this second series, it was observed that Red, on the anesthetized side, even within 5 minutes had a marked weakening of the conditioned reflex, when the bell rang. By the 10th minute the use of the positive stimulator (m_{120}) led to a marked diminution of salivary secretion on both sides. Differentiation almost did not change, being slightly weaker on the side opposite to the anesthesia. Within 20 minutes the salivary secretion from the conditioned reflex stimulation was fully restored on the anesthetized side while remaining depressed and below the usual by the 30th minute on the opposite side. The behavior of the dog while within the chamber remained unaltered, food being well consumed.

In Elsa anesthesia of the oral mucosa with 10% Dicaine produced sharp changes in the activities of the parotid glands when responding to conditioned reflexes. At the beginning, at the 5th minute, salivary secretion was markedly depressed on the opposite side while remaining quite large on the anesthetized side. By the 10th minute, the activities of both glands were markedly altered undergoing ultraparadoxical and paradoxical phases. The secretory response to the positive stimulator (m_{120}) was totally supressed while the response to the negative stimulator (m_{60}) was one of copious salivation. Also, the weak stimulator (light) produced a greater conditioned reflex response than the powerful stimulators.

The behavior of the dog while within the chamber was also changed very sharply as it would eat only with the action of a weak stimulator-light. When powerful stimulators acted, the experimenter had to be present before the dog would eat. However, by the end of the experiment (30th minute) the powerful stimulators caused it to eat voraciously on its own.

In Wolf anesthesia with 10% Dicaine caused a gradual extinction of the response to the conditioned reflex stimuli, both left and right sides being affected.

Thus, it seems that exclusion of some of the afferent receptors by means of anesthesia of a portion of the oral mucosa produces changes in conditioned reflex salivary secretion: the stronger the anesthesia, the greater the changes in observable salivary secretion.

The type-specific characteristics of the nervous system possessed by the individual animals seem to have great significance in the changes which are produced. In a dog of the feeble type even 5% Dicaine produced considerable alteration in the conditioned reflexes of both the left and right sides. In a dog of the strong, unstable type 5% Dicaine altered salivary secretion on the anesthetized side, while still stronger anesthesia (and, in all probability, by its action through the blood stream) could not be counterbalanced by the nervous system so that conditioned reflex activity changed on the right side as well as the left. In an animal of the strong,

stable type 5% Dicaine produced an effect only on the anesthetized side. It required a 10% solution of Dicaine to affect the opposite side also, although not profoundly, the changes having a quantitative character only.

In analyzing the various mechanisms causing changes in the salivary secretion of the parotid glands by the application of the Dicaine to the oral mucosa, it is essential to remember that the anesthetic might be acting directly by reason of being absorbed into the blood stream. If that were so, the changes should be the same for both parotid glands, right as well as left. Yet we always observed an asymmetrical response in the working of the parotid glands and in a number of instances there was seen a marked diminution of salivation on the anesthetized side.

The data we have accumulated seem to demonstrate that afferents stimulated in the oral mucosa of the mouth exert an important influence upon the functional state of nerve centers present in the right as well as the left cerebral hemispheres and, therefore, aid in determining salivary secretion on both sides whether either conditioned or unconditioned reflexes are employed in determining an answer to this question. This conclusion is aided by our studies with partial searing of the oral mucosa [3]. The presence of additional stimuli (focus of inflammation) going centrally from the oral mucosa, as well as exclusion of the usual stimuli, both lead to changes in the activities of the paired parotid glands. With all this, we have additional data which testify to the fact that the degree of stimulation of both the right and left cerebral hemispheres depends not only on the stimuli coming from the oral mucosa but also is influenced by other internal and external alterations of the environment of the organism.

SUMMARY

Dogs having paired parotid gland fistulae and studied for three years by the investigator were used to observe the alterations in the conditioned reflexes caused by anesthesia of a portion of the oral mucosa. It was concluded that the basic type characteristics of the dogs used had much to do with the response obtained.

Dogs of the strong type had inhibition on the anesthetized side only when 5% Dicaine was used. In dogs of the weak type the conditioned reflexes were inhibited on both sides. With stronger anesthesia the changes observed were greater. Dogs of the unstable type manifested both an equalizing and a paradoxic phase. As with the 5% Dicaine, use of the 10% Dicaine in dogs of the weak type produced abolition of the conditioned reflexes on both sides.

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EFFECT OF MUSCULAR WORK ON THE MOTOR FUNCTION OF THE STOMACH AND DUODENUM

A. N. Pomeltsov

From the Laboratory of the Physiology and the Pathology of Digestion (Chief – Doctor of Med. Sci. S. I. Filipovich) in the Institute of Normal and Pathological Physiology Acad. Med. Sci. USSR (Director – Corresponding Member of the Acad. Sci. USSR, V. N. Chernigovsky)

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Despite the considerable theoretical and practical significance of studies directed to the clarification of the effect exerted by muscle work upon the activities of various organs and systems in the healthy organism, the changes in the digestive tract due to muscle work have been studied but inadequately. The overwhelming majority of the studies have dealt with changes in the secretory glands under the influence of the most varied kinds of muscle work. In regard to gastrointestinal movements the studies have been few and have dealt principally with the function of stomach emptying.

Observations on the influence of static effort upon motor (peristaltic) activity of the digestive tract [2, 5] are very scant and deal only with stomach movements, particularly under conditions of physiological hunger.

The present study is devoted to an examination of the influence static muscular effort exerts upon three different types of gastric motor activity: periodic, "hunger" contractions, "acid" movements [6] and the peristaltic movements occurring when a stomach has been filled with food. It was interesting to compare simultaneously motor activity of the duodenum with changes in the motor activity of the stomach.

The results we obtained enabled us to compare the influence of static motor effort upon gastric and duodenal motility with results obtained when making analogous studies upon the influence of dynamic motor effort (running in a treadmill) in the same animals [4].

EXPERIMENTAL METHODS

The experiments were conducted using 6 dogs, each one having a fistula in the fundic portion of the stomach. Besides this, three of them had duodenal openings to the outside. The animals were used in these experiments after they had fasted 18-20 hours. The experiments were begun in the absence of a pronounced acid secretion (of the stomach contents). The usual balloons recorded gastric movements. In order to record duodenal activity, a modification of this method was used, as worked out in our laboratory by V. F. Mostun [3].

"Acid" movements of the stomach were initiated by sham feeding (feeding with the gastric fistula open); within 5-7 minutes, 150 grams of raw meat were given. Experiments made to observe gastric peristalsis during digestion were conducted in an identical manner except that the sham feeding was replaced by the real.

The static muscular effort consisted in having the dog support a large weight (sand bag) which was laid across the shoulder girdle of the animal. The intensity and duration of the load was varied in two ways: 1) relatively short but very intense effort; in this instance, the dog being made to support a weight equal to its own (sometimes more) for 10 minutes; 2) prolonged but less intense effort; the weight being in this instance about half the body weight and being supported for about an hour.

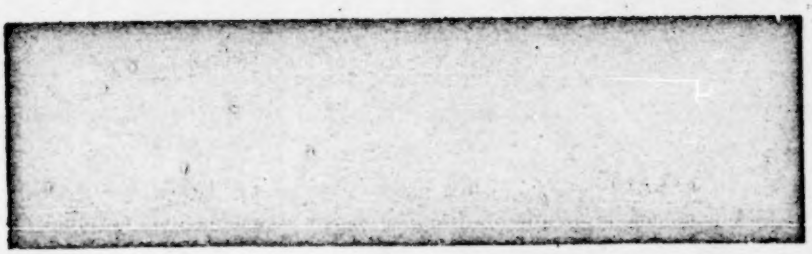

Fig. 1. Influence of intensive static effort upon "hunger" contractions of the stomach (dog Kashtan).
Read from left to right, from above down. Arrows indicate beginning and end of the static work.

Before commencing experiments pertaining to the effect of muscular loading, "background" studies were made and the influence of the new type of environment, associated with the loading studies, was extinguished.

The influence of high intensity, short duration static effort upon "hunger" contractions of the stomach was observed in two dogs – Kashtan and Tsirkach. In these experiments, the "background" period of "hunger" contractions was recorded first, after which the next period of contractions was preceded by the placing of the static load. Figure 1 represents a typical experiment (dog Kashtan).

EXPERIMENTAL RESULTS

It is evident that a static load, placed at the very beginning of a period of "hunger" contractions, depresses them in a very short interval of time. Removal of the static load allows the "hunger" contractions to resume. The same relationships were observed in analogously conducted experiments with a second dog – Tsirkach, although a somewhat larger load was required.

The influence exerted by prolonged but less intense static loads upon "hunger" contractions occurring periodically in the stomach and duodenum was studied experimentally on two dogs – Reke and Peggy. The experiments were conducted in two variations. In the first variation, after having recorded the "hunger" contractions and in the quiet interval, the static load was placed and kept to the end of the next period of contractions. When this maneuver was employed, it was evident that these latter contractions began and ended (at the termination) of an hour of static effort. The majority of these experiments indicated that the contractions of the "hunger" type in the stomach occurring during static effort were of shorter duration; the analogous duodenal motor changes were also less pronounced.

In the second variation, work of the same intensity and duration, was stopped just before an anticipated period of gastric "hunger" contractions. When this was done, the gastric and duodenal contractions were not depressed in regard to the length of time, number or intensity of the effort.

The influence of static loading was studied upon the digestive and "acid" gastric contractions of dogs Kashtan, Tsirkach, Peggy and Aster; the amount of load and length of time it was kept on was the same as in the preceding experiments.

Figure 2 is a typical sample. It can be seen that "acid" (Fig. 2,a) and digestive movements of the stomach (Fig. 2,b) are not markedly depressed by a short and intensive period of static loading. There was no depression of digestive movements of the stomach under the influence of the second type (prolonged) of static loading. However, when the load was increased in the same animals, it could be seen that a very intense static load did produce some depression of gastric motility (Fig. 2,c).

In order to compare the motor changes in the stomach with those in the duodenum, experiments were conducted on three dogs – Aster, Dik, and Peggy – intensive static loading being observed for its effects upon the periodic "hunger" contractions of both bowel segments. Figure 3 represents such an experiment on dog Aster. It can be seen that the braking effect of the static loading is clearly reflected in gastric movements but not in

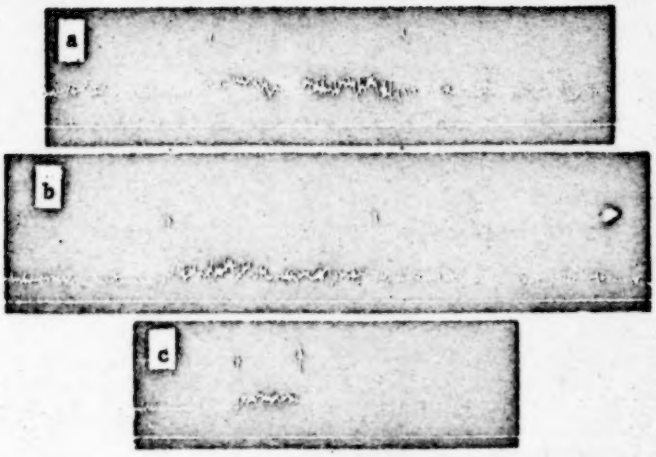

Fig. 2. Influence of intense static effort on "acid" (a) and digestive (b,c) movements of the stomach (dogs Kashtan and Aster).
Read from left to right. Arrows indicate beginning and end of static effort.

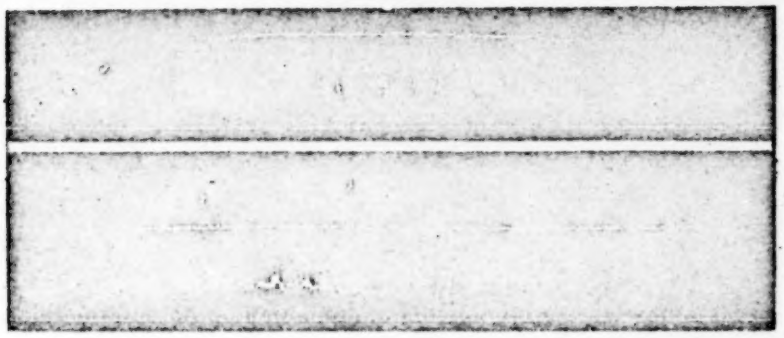

Fig. 3. Influence of intense static effort on "hunger" contractions of stomach and duodenum (dog Aster).
Read from left to right; meaning of tracings (from above downward): duodenal peristalsis; gastric movements; time marker. Arrows indicate beginning and end of static load.

those of the duodenum. The other animals studied gave about the same response, i. e., a more or less clearly seen difference between the gastric and duodenal responses to static loading. Thus, it can be said, that when static work depresses "hunger" contractions of the gastrointestinal tract the effect is more manifest in the stomach than it is in the duodenum. Static loading alters the "hunger" gastric contractions more sharply than it does the "acid" and digestive movements.

It is materially important to observe that static loads exert a depressing effect on the gastrointestinal tract only while it is being exerted, as its effect disappears almost as rapidly as the effort ceases, this being different from the effects of dynamic efforts [4] where the same animals demonstrated a braking motor effect not only during the effort but also for some time (minutes) after its cessation. Analysis of our data shows that short, intensive static loading powerfully depresses the motility of both the stomach and duodenum. The results of the second variant of out experiments when there was prolonged static loading of a lesser intensity should be compared:

in this instance the depressing effect of the static loading vanishes as quickly as the load is removed; in the period of "hunger" contractions which begin immediately after the load is removed, no braking can be observed. In both cases the rapidity and clear-cut sharpness of the effect indicate a reflex mechanism as the initiator of the changes.

Special tests established the fact that neither the new experimental conditions associated with the performance of the muscular effort nor the changes in the intra-abdominal pressure accompanying the muscular contractions can possibly be the source of the braking effect we observed during muscular work.

It can be supposed that the static work accomplishes its braking effect by means of reflex pathways originating in the moving tissues, first of all by a reflex involving the receptors of the moving muscles. As a proof of this, we can accept the studies of V. I. Beltukov and M. R. Mogendovich and their associates who demonstrated experimentally that adequate stimulation of muscle receptors suppresses gastric peristalsis. We can think that the stream of impulses coming from taut muscles creates a stimulatory focus in the area of the movement analysor which, in its turn, affects other nerve centers including the digestive, regulating gastrointestinal activities including the motor functions.

This question we explored in special experiments. As an indicator of changed irritability of the digestive center we used unconditioned salivary secretion and movement responses to specific stimulation by food. These studies were made on the same dogs, the static loads being of same intensity and duration. As a result we observed that, during intensive static work, the desire to eat was sharply diminished; the animal usually refused food. This effect of static loading disappeared as quickly as the load was removed, salivary secretion being noted very frequently just as the burden was taken off. Loss of appetite was observed also when static loads of lesser intensity but longer time were applied, this being manifested by the animals not making a vigorous movement to approach the food at the end of the hour of static labor, however, this reaction tended to vanish with the removal of the load.

These observations testify to the manner in which static loading affects the digestive center.

Finally, we should note the differences between the way static loading affects the periodic "hunger" contractions and "acid" movements, those arising during sham feeding in connection with varying functional states of the digestive apparatus and the condition of the central nervous system.

The entire discussion compels us to believe the changes in motor activity accomplished as a response to static effort are mediated by a basic reflex mechanism which must work in close conjunction with alterations in the functional state of the digestive center.

SUMMARY

The effect of muscle work on the gastric and duodenal motor function was studied in dogs.

It was observed that the static stress inhibited the "hunger" contractions of the stomach and duodenum; the inhibitory effect disappeared as the stress ceased. The static muscle work effect was more pronounced in "hunger" contractions of the stomach than in those of the duodenum. Under the influence of static stress, the periodic "hunger" contractions of the stomach varied more extensively than its "acid" and digestive contractions.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

THE ROLE OF THE TELENCEPHALON IN COMPENSATORY PROCESSES IN DUCKS

V. N. Drozdova

Physiologic Laboratory of the Academy of Sciences USSR (Director – Corresponding Member of the Academy of Sciences USSR E. A. Asratyan), Moscow

(Received January 9, 1956. Submitted by Active Member of the Academy of Medical Sciences USSR P. K. Anokhin)

The study of compensatory adapations of the nervous system in animals generally and in birds in particular is of certain theoretical and practical interest. Much work has been done on this by E. A. Asratyan and his collaborators (V. A. Dmitriev, A. I. Karamyan, B. D. Stefantsov and others); the experimental animals used were pigeons almost without exception. At present Asratyan's collaborators are studying these phenomena on birds (hens and ducks) in whom walking is more pronounced and which are therefore more suitable for these particular investigations.

The problem posed was to discover the role of the cerebral hemispheres in birds in compensatory phenomena developed following transection of the posterior half of the spinal cord.

It was necessary to establish concretely the following: 1) characteristic dynamic features of disruption and restoration of function in ducks following transection of the posterior half of the spinal cord, 2) the role of the higher sections of the central nervous system, in particular the cerebral hemispheres, in the compensatory phenomena accompanying such transection.

EXPERIMENTAL METHODS

Extensive experiments were staged. First the posterior half of the spinal cord was transected at the level of the 6th thoracic vertebra, then after complete compensation of disturbed functions both cerebral hemispheres were extirpated, successively. The operations were carried out under urethane-novocaine narcosis and under strictly aseptic conditions.

For more complete characterization of the disturbance and restoration of function all the ducks were subjected pre- and postoperatively to measurements of the flexor reflex threshold (induction stimulation), to careful visual observation of the course of recovery of motor and sensory function of the wings and legs and to measurements of the skin temperature of the feet.

EXPERIMENTAL RESULTS

Transection of the posterior half of the spinal cord as performed on 25 ducks led to profound disturbances of sensory-motor and trophic functions. During the first 4-5 days the ducks remained lying on the side or prone with extended legs and neck (Fig. 1). Reflexes from the wings and legs were sluggish; crossed reflexes were difficult to elicit and then only in rare cases. The threshold for flexor reflexes to induction current rose to 1.5-2 cm distance between the coils of the Du Bois-Reymond apparatus(the 5,000 turn coil was fed from a 2 v accumulator) (Table 1).

TABLE 1

Flexor Reflex Thresholds in Duck No. 11 on Section of the Posterior Half of the Spinal Cord (in cm of intercoil distance) Operation February 19, 1952.

Limb	Before operation			After operation								
	2/2	2/15	2/18	2/21	2/23	2/27	3/1	3/7	3/10	3/14	3/30	4/10
Right	16	16	16	14	14	14.5	14	13.5	13.5	14	15	15
Left	17	16	16	14.5	14	14.5	14.5	13.5	14	14	14.5	15

The skin temperature of the feet also showed a certain rise; diminution of tonus was noted in the limbs. The ducks ate and drank without assistance. In addition to the disturbances mentioned there were also trophic changes, namely dryness and coarsening of the skin on the plantar surfaces of the feet. In 6 ducks loss of voice was observed.

The disturbed functions began to be compensated gradually and rather slowly. Thus, from the 4-5th day the ducks began to hold up the head, and the reflexes from wings and legs became more brisk. On the 5-6th

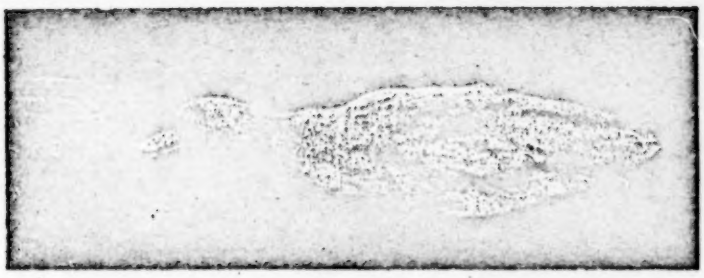

Fig. 1. Duck No. 9 on 2nd day after transection of the posterior half of the spinal cord.

day they were no longer prone, but sat up. The legs, however, remained more often extended. On the 7-8th day the ducks could raise themselves and stand at the side of the box on widely spread legs, on the 9-12-15th day they started making attempts at locomotion; at first they made 1-2-3 steps on semiflexed legs, then fell or stopped. It was interesting to note that on loss of equilibrium the ducks often made use of wings and tail for support. Towards the 15-20-25th day the ducks began to walk, at first slowly, then more quickly, but still limping and often stumbling. These disturbances became especially obvious on fast walking and running, as well as on negotiating obstacles which was a particularly difficult problem for the experimental birds, even two, and sometimes more, months postoperatively when superficially the compensation of function appeared to be complete.

After 2-3 months full recovery of the disturbed functions was seen and the operated ducks could not, usually, be distinguished from healthy ones. The very slow recovery of function, disrupted by transection of the posterior half of the cord, speaks for the important role played by acquisition of skills in these compensatory phenomena.

To elaborate this further, however, it was necessary to remove the cerebral hemispheres in these experimental birds.

With this aim in view 9 ducks with already well-compensated functions following transection of the posterior half of the spinal cord were subjected to right hemispherectomy. Two ducks died several days after this procedure. The 7 surviving ducks were subjected to left hemispherectomy, 2-3 and in some cases 4 months later.

The following results were obtained. Removal of one cerebral hemisphere led to decompensation of all those impairments of function observed after the spinal operation: profound disturbances of sensory, motor and trophic functions developed. In the first 4-6 days the ducks remained lying, most often prone, with extended legs (as was the case after the spinal operation). Contralateral hypotonia was noted in the muscles. Reflexes could be elicited from both legs and wings but they were sluggish on the side opposite to the removed cerebral hemisphere. The flexor reflex threshold on the contralateral side rose to 1.5-2.5 cm intercoil distance, and there was considerable increase in the skin temperature – by 4-6° on the average (Table 2).

TABLE 2

Flexor Reflex Threshold (Legs) in Duck No. 11 on Removal of Right Cerebral Hemisphere in cm Inter-Induction Coil Distance. Operation October 20, 1952.

Limb	Before operation			After operation						
	10/11	10/14	10/17	10/22	10/25	10/27	10/30	11/14	11/28	12/13
Right	15,5	15	16	14.5	15	15	16	16	16	15.5
Left	16	16	15.5	12.5	13.5	15	15	15.5	15.5	16

Following the first hemispherectomy (right) gradual restoration of the disturbed functions occurred in time. For instance the flexor reflex thresholds returned to normal on the 5-6th day in most cases.

In some ducks restoration of normal sensation in the limbs was delayed as long as 20-25 days. Restoration of skin temperature took, as a rule, longer and in isolated cases it failed to reach normal levels in the course

Fig. 2. Duck No. 9 on the 20th day following right hemispherectomy.

Fig. 3. Duck No. 9 on the 10th day after removal of second cerebral hemisphere (left).

of 2 months. Five ducks could stand on the 6-10th day, the other 2 – on the 18-22nd day postoperatively (Fig. 2). Normally coordinated walking was restored in 4 ducks on the 35-45th day, in 3 – on the 20-25th day.

Extirpation of the second (left) hemisphere was performed on 7 ducks in which right hemispherectomy had been followed by complete restoration of decompensated functions. It must be noted that this operation was much more grave. Only because of the specially developed nursing and feeding procedures could the operated ducks survive.

Five ducks were available for observation following the removal of the second hemisphere. In the first few days decompensatory phenomena were observed (as was the case after the first two surgical procedures). The ducks were lying prone with extended legs and neck unable to hold up the head. They did not eat or drink spontaneously and did not react to their surroundings or stimulation. Hypotonia of all muscles was seen. The flexor reflex threshold and skin temperature of the limb contralateral to the hemisphere removed last were raised.

Marked loss of weight was observed despite fully adequate diet. Lubrication of the feathers with coccygeal gland secretion ceased, resulting in a ruffled appearance.

In time the condition of the birds began to improve slowly. Beginning with the 9-12th day the ducks started to raise themselves on their feet and to stand (Fig. 3). The flexor reflex thresholds to induction current became normal on the 8-10th day. Differences in skin temperature were fairly slow to disappear, as was also noted after the first hemispherectomy. It must be remarked that once the ability to stand was recovered the ducks tended to exhibit "persistent" standing. They remained standing most of the time with their legs wide apart; they sat only rarely and almost never lay down, often sleeping in the standing position. Later on this inert state alternated with periods of increased automatic movements. The birds could not stand still at all, continuously cleaning themselves with the beak or feet, and twitching one or other wing all the time. After $1^1/_2$ months following removal of both cerebral hemispheres the ducks began slow locomotion on flexed and widely spread legs. In time the gait became more normal but there was no complete recovery of walking ability.

The data presented above permit the conclusion that the cerebral hemispheres in ducks participate in the compensation of derangements caused by section of the posterior half of the spinal cord. This participation is not, however, of such paramount importance as in mammals.

SUMMARY

Extensive experiments were carried out on ducks. It was noted that transection of the posterior half of the spinal cord in ducks caused profound sensory-motor and trophic disorders. Gradually the disturbed functions were restored, this time more quickly than after the operation on the spinal cord. Ablation of one hemisphere led to decompensation of all the recovered functions. In the course of time the disturbed functions were again restored, this time more quickly than after the operation on the spinal cord. Ablation of the second hemisphere in the same ducks was followed by profound recurrent disturbances which disappeared slowly and were not fully compensated.

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HORMONAL INFLUENCES ON COMPENSATORY RENAL HYPERTROPHY

V. V. Osipovich

Chair of Physiology (Chief – Corresponding Member of the Academy of Medical Sciences USSR A. G. Ginetsinsky), Novosibirsk Medical Institute (Director – Professor G. D. Zalessky), Novosibirsk

(Received September 19, 1956. Submitted by Active Member of the Academy of Medical Sciences USSR L. A. Orbeli)

Among those factors which exert trophic influences on renal tissue the most prominent is that of adenohypophysis. Following hypophysectomy marked reduction in the weight of the kidneys is noted and there is equally marked reduction in all the quantitative indicators of renal function [6, 8]. If a hypophysectomized animal is subjected to unilateral nephrectomy no compensatory hypertrophy of the remaining kidney is observed [8, 3].

Less clear is the importance of other trophic factors. Complete denervation of the kidney in no way affects its ability to undergo compensatory hypertrophy [1, 7]. In castrated animals and those with removed thyroid glands unilateral nephrectomy is followed by hypertrophy of the remaining kidney. However, administration of testosterone and of thyroxine accelerates the development of the hypertrophy [4, 5, 9].

During investigation of the influence of dietary factors on compensatory renal hypertrophy the present author noted greater increase in the weight of the remaining kidney during the winter as compared with hot summer weather. This led to special studies on the relation of compensatory hypertrophy and hormonal influences exerted by the thyroid and the hypophysis, since it is known that the activity of these endocrine glands is enhanced by low temperatures and depressed by high temperatures.

EXPERIMENTAL METHODS

Experiments were carried out on sexually mature white rats (29 animals). Male rats of the same age were taken for each series. Five days prior to the beginning of experiments the rats were put on a standard full diet [2]. 15 days prior to operation the animals were placed under appropriate conditions of temperature and were maintained in them until the end of the experimental period. The environment for "cold" rats varied from 8° to 10°, for "warm" ones from 25° to 30°. The right – control – kidney was removed through a posterior-lateral incision. 20 days later the remaining kidney was removed and compared by weight with the control one. The weight of the kidneys was calculated in absolute units. Renal tissue hypertrophy was expressed in percentage of the increment in the absolute weight of the kidney.

The kidneys and thyroid glands of these rats were subjected to histologic treatment and microscopic examination.

Experimental results are given in Tables 1 and 2.

As can be seen from these tables, renal hypertrophy is more marked in the "cold" rats. It averages 28.7% with limits of fluctuation from 16 to 41.2%, while in the "warm" group the average is 17.4% with limits of fluctuation from 13.1 to 21.7%.

In order to clarify the question concerning the significance of enhanced thyroid activity in this "cold" effect a series of experiments was staged in which thyroxine and methylthiouracil were administered to

TABLE 1

Renal Hypertrophy in Low Temperature Environment

Weight of removed kidney (in mg)	Weight of remaining kidney (in mg)	Wt. increase hypertrophied kidney % of control
1490	2010	34.9
1280	1560	21.9
1000	1290	29.0
1120	1300	16.0
1310	1850	41.2
1065	1430	34.2
970	1200	23.7
Average value of increment		28.7

TABLE 2

Renal Hypertrophy in High Temperature Environment

Weight of removed kidney (in mg)	Weight of remaining kidney (in mg)	Wt. increase hypertrophied kidney % of control
1310	1560	18.2
1250	1430	14.4
900	1080	20.0
920	1120	21.7
990	1185	19.5
985	1130	14.7
910	1030	13.1
1035	1335	17.6
Average value of increment		17.4

nephrectomized rats. Both substances were introduced by tube into the stomach. Methylthiouracil was administered as a 3% aqueous suspension, 30 mg per 100 g weight, thyroxine – 120γ per 100 g weight. Thyroxine was given daily from the day of the operation, whereas administration of methylthiouracil was begun 20 days prior to nephrectomy. The rats were kept at a temperature of 8-12°.

Results of these experiments are summarized in Table 3.

TABLE 3

Increment in Weight of Remaining Kidney in Percentage of Contol

In control group*	Rats given thyroxine	Rats given methylthiouracil
66.6	63.3	111.2
42.8	103.6	132.8
68.4	69.1	104.3
80.9	50.6	110.4
51.2	71.7	—
Average values: 61.9	71.6	114.7

* The greater degree of hypertrophy of the remaining kidney as compared with data in Table 1 is explained by the fact that in the present series the rats were young (average weight 176 g) while in the previous series the rats were old (average weight 260 g).

Table 3 shows that while thyroxine did exert some effect on the development of compensatory renal hypertrophy it was much less clear than the effect of methylthiouracil. It is very probable that the latter could be explained by the well-known enhancement of thyrotropic hormone secretion under conditions when thyroid function is suppressed. The possibility is not excluded, however, that this is accompanied by increased output of other renotrophic factors by the hypophysis.

SUMMARY

In animals kept in the cold, compensatory hypertrophy of the kidneys after unilaterial nephrectomy was considerably greater than in animals kept in warmth. It is quite probable that the effect of cold depends on the intensified secretion of the thyrotropic hormone by the hypophysis, because the introduction of methylthiouracil, which increases the secretion of this hormone, was accompanied by a considerable increase of compensatory renal hypertrophy.

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INCREASED SENSITIVITY OF TISSUES TO STIMULI IN AN AUTOTRANSPLANTED LIMB

E. V. Gurova

Scientific Research Institute of Experimental Surgical Equipment
(Chief – M. G. Ananiev), Ministry of Health of the USSR

(Received June 3, 1955. Submitted by Active Member of the Academy of Medical Sciences USSR V. N. Chernigovsky)

Changed reactivity of the tissues in an autotransplanted organ, especially in the first few months following the transplantation, can be apparent not only with respect to electric current stimulation (1) but also to chemical stimuli, including pharmacologic substances.

It is well known that denervated structures exhibit enhanced sensitivity to chemical stimuli. This is explained by lowering of the sensitivity threshold of denervated striated muscle to current as well as to adrenaline, acetylcholine, pilocarpine and other chemical substances [2] and also by metabolic disturbances and structural changes in proteins accompanying denervation of an organ [3].

The aim of the present investigation was to elucidate the degree and nature of altered reactivity of tissues in autotransplanted limb in dogs to administration of pharmacologic substances possessing different mechanisms of action; comparison was made of these changes in relation to different intervals of time following the operation•.

EXPERIMENTAL METHODS

Of substances with a cortical mechanism of action, sodium bromide, which enhances the inhibitory functions of the cortex, and caffeine, which increases the excitatory processes were tested. Acetylcholine and adrenaline were taken for comparative studies.

Sterile 5% solution of sodium bromide was given intravenously (10 mg per 1 kg body weight). Also intravenously was given a sterile 10% solution of caffeine, always in the amount of 3 ml for dogs weighing 12-15 kg.

Acetylcholine and adrenaline were administered subcutaneously: 0.015 g of the former and 2 ml of the latter in sterile physiologic solution 1:1,000.

The function of the transplanted limb was studied prior to administration of the substances and during $1^1/_2$-2 hours following their administration.

In order to trace the dynamics of the action of a given substance on the motor nerves and muscles of the limb changes in the indicators were recorded at 5-7 minute intervals by means of chronaxie measurements.

The reactivity of the tissues of the autotransplanted limb was compared with the reactivity of the healthy limbs in the same dogs. Five experiments were carried out on each dog: 1 control, the rest with administration of adrenaline, caffeine, acetylcholine and bromide. Intervals between experiments were 6-7 days.

• The operations were performed by N. P. Petrova of the Surgical Department.

The experiments were performed on 20 dogs, nine of which were operated (autotransplantation of the left hind limb), while the remainder constituted a control group of healthy dogs. Altogether 120 experiments were performed, with over 11,000 separate measurements of the rheobase and chronaxie for motor nerves and muscles of healthy and autotransplanted limbs in dogs.

EXPERIMENTAL RESULTS

Introduction into the organism of pharmacologic substances exerted a general effect on the animals. Bromide, as a rule, produced drowsiness and sleep, with changes in the motor nerve and muscle rheobase and chronaxie. In experiments on healthy dogs administration of sodium bromide increased the rheobase in 47.2% of cases, in 32.6% the threshold of motor excitability changed within the limits of the original values and in 20.2% of cases it even showed a decrease. Motor nerve chronaxie was usually increased under the influence of bromide (84.6%).

Experiments with caffeine showed decrease of rheobase and increase of chronaxie of the motor nerves. Under the action of acetylcholine motor nerve rheobase and chronaxie were decreased $1^1/_2$ times more frequently than in control experiments, while chronaxie was more often increased.

Changes in the rheobase and chronaxie of the limbs did not extend beyond the limits of normal. Rheobase changes were within the range of 1-30 v, chronaxie – 0.01-0.3μ F. More frequent lowering of motor excitability threshold (rheobase) was characteristic for caffeine and adrenaline, while for bromide and acetylcholine more frequent increase in tissue chronaxie was characteristic.

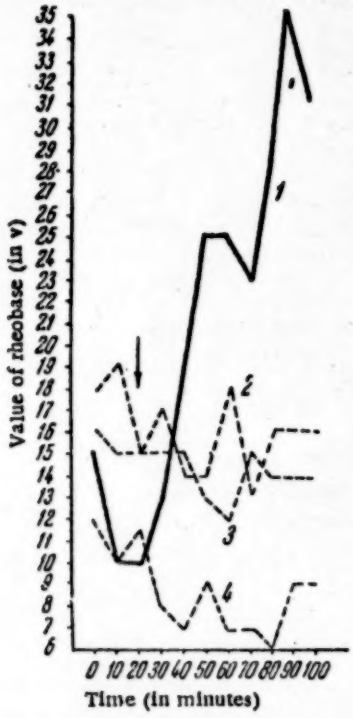

Fig. 1. Changes in limb muscle rheobase following administration of adrenaline; dog Orion.
1) autotransplanted limb; 2), 3), 4) healthy limbs. Arrow indicates moment of adrenaline injection.

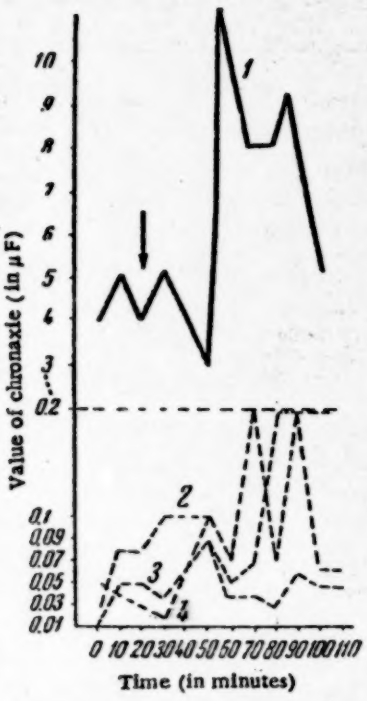

Fig. 2. Changes in limb muscle chronaxie following administration of adrenaline; dog Lisa.
Records similar to Fig. 1.

Greater sensitivity to pharmacologic substances was noted in autotransplanted limbs.

In the case of the dog Orion administration of pharmacologic subtances 12-21 days postoperatively caused almost no changes in the rheobase and chronaxie of the healthy limbs. In the operated limb chronaxie of the denervated muscles increased from 5 to 8 μF on introduction of bromide, and dropped from 7 to 4 μF on introduction of caffeine. Acetylcholine increased the rheobase from 9 to 25 v and lowered the chronaxie from 7 to 3 μF. Adrenaline produced a sharp increase in the devernated limb rheobase (Fig. 1).

In the case of the dog Lisa experiments were carried out 16-45 days following operation. Administration of bromide changed the rheobase of the healthy limbs from 22 to 15 v, of the operated limb – from 12 to 14 v. The muscle chronaxie of the healthy limbs showed a transient increase from 0.03-0.09 to 0.2 F; in the case of the operated limb it showed a persistent increase from 3 to 7μ F. Caffeine produced a slight increase in the chronaxie of the healthy limbs and an increase from 4 to 7μ F in the operated limb. Administration of acetylcholine produced no changes in the healthy limbs but decreased the chronaxie of the operated limb from 8 to 5μ F. Particularly marked effect was exerted by adrenaline on denervated muscle: the already high chronaxie increased from 5to 10μ F following administration of adrenaline and continued to increase beyond the limits of its determination (Fig. 2).

In the dog Druzhok administration of caffeine did not alter the rheobase but decreased the chronaxie of the denervated muscles from 9 to 3μ F, while administration of adrenaline raised it from 3 to 7μ F; the chronaxie of the healthy limbs changed at the same time within the limits of original values. In the dog Seraya, $1^1/_2$ months after operation acetylcholine and adrenaline injections caused increases of the rheobase of the transplanted limb from 20 to 65 v and increase of muscle chronaxie from 4 to 10μ F and higher. No such reactions of the left hind limb muscles were observed in these dogs prior to autotransplantation.

Such heightened reactivity of transplanted limbs is rarely encountered at later periods of the graft's "taking".

Tissue Sensitivity in Healthy and Autotransplanted Hind Limbs in Dogs to Pharmacologic Agents (Average Data) in Percentage of Original Values

Pharmacologic Agent	Rheobase in V		Chronaxie in μF	
	healthy limb	Autotrans-planted limb	healthy limb	autotrans-planted limb
Bromide	230	250	100	600
Caffeine	65	45	100	100
Acetylcholine	200	175	70	600
Adrenaline	20	250	100	210

The dogs Silva and Lisa showed, 8 months postoperatively identical reactions to administration of bromide, caffeine, acetylcholine and adrenaline in the transplanted and healthy limbs. The dog Belyi, however, showed increased reaction to these substances after a similar interval.

The table gives average values for maximal tissue reaction to pharmacologic substances as percentages of values obtained from the same limb prior to administration of these. Judging by the rheobase, for example, tissue reactivity in the transplanted limb to adrenaline is 12 times greater than in the symmetrical healthy limb.

Chronaxie values indicate that the reactivity of the tissues in the transplanted limb to bromide and acetylcholine is increased 6 times compared with the healthy limb.

Thus increased, and at times altered, reactivity of tissues in autotransplanted limbs to adrenaline, acetylcholine, caffeine and bromide has been established. This heightened tissue reaction subsides as innervation is restored.

SUMMARY

120 experiments were made on 20 dogs to study the effect of adrenalin, acetylcholine, caffeine and sodium bromide (subcutaneous and intravenous) on the rheobase and chronaxie of motor nerves and muscles in healthy and autotransplanted limbs. The sensibility of denervated tissues towards the mentioned agents was shown to be increased and of a different nature; it decreased as the tissues' innervation recovered.

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MECHANISM OF RENAL INVOLVEMENT IN THE PATHOGENESIS OF EXPERIMENTAL NEUROGENIC HYPERTENSION

COMMUNICATION II. CORRELATION OF RENAL CIRCULATION AND RENAL RENIN CONTENT IN EXPERIMENTAL NEUROGENIC HYPERTENSION AND FOLLOWING DENERVATION OF THE KIDNEYS

M. Ya. Ratner and R. S. Eisenhardt

From the team of Full Member of the Academy of Medical Sciences USSR Professor M. S. Vovsi and Laboratory of General Physiology, Institute of Normal and Pathological Physiology (Director — Full Member of the Academy of Medical Sciences USSR V. N Chernigovsky), Academy of Medical Sciences USSR, Moscow

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According to widely held views the cause directly responsible for increased production of renin during the development of hypertension is progressive renal ischemia. This view is based on the fact that hypertension caused by constriction of renal arteries (so-called "Goldblatt hypertension") is accompanied by enhanced renin secretion and a rise in renin content of the kidneys [15, 19].

V. N. Chernigovsky and collaborators developed a new concept in a series of investigations [4, 5, 6, 8, 9] which postulates that in the courseof development of neurogenic hypertension, formation of renin in the kidneys is stimulated by the nervous system independently of changes in renal blood flow.

The present work was concerned with direct elucidation of the significance and the role of renal ischemia in the mechanism of renal pressor factor involvement and more especially of the renin system in experimental neurogenic hypertension.

With this aim in view a study was made of renal circulation both during "inclusion" of the renal pressor factor in experimental neurogenic hypertension and during its "exclusion" by means of denervation of the kidneys.

EXPERIMENTAL METHODS

Experiments were carried out on rabbits weighing 1.8 to 2.3 kg.

Induction of hypertension, measurements of arterial blood pressure, denervation of the kidneys and determination of renin content of the kidneys were performed in ways similar to those described in previous papers by one of the authors [4, 5].

Two criteria were used in determining the state of renal circulation: renal blood flow and the so-called filtration fraction, whose value represents the ratio of filtration to plasma flow and gives an idea of the relative tonus of afferent and efferent blood vessels.

Renal blood flow was determined by Diodrast clearance coefficient, glomerular filtration by endogenous creatinine clearance coefficient. Clearance coefficients for these substances, according to modern data, represent most nearly the state of the respective renal functions in the rabbit [22]. For the sake of greater precision the investigation was carried out under conditions of practically constant blood levels of Diodrast. This was achieved by continuous drip, maintained at a constant rate. Sterile Diodrast solution, 80 mg% in physiologic solution, was

sed for the injections.

The animal was immobilized on a table in the supine position. A needle connected in the usual manner with a vessel containing Diodrast solution was inserted into the pinna vein and the drip started. The rate of 1-1.5 ml per minute was maintained by means of a screw clamp situated above the dropper.

Preliminary administration of Diodrast designed to create the necessary blood level was continued for 30 minutes. At the beginning of this period the femoral artery was exposed and ligated.

In each experiment determination of renal function was, as a rule, performed over three periods of time, each 13 to 35 minutes in duration. The urine excreted during each of these periods was collected by catheter; the bladder was then washed out with 50-60 ml of warm water which was aspirated by a syringe. Blood was taken from the femoral artery in amounts of 5-6 ml, in one part of the experiments by means of a cannula, in another - by gravity.

Chemical estimations of blood and urine Diodrast were carried out by the Alpert [11] method; creatinine was estimated by one of the modifications of Folin's method [17].

Clearance coefficients were calculated in the standard way. The described procedure involved fairly considerable (for rabbits) withdrawals of blood, which made it necessary to obtain evidence excluding changes of blood pressure during the investigation itself. For this purpose repeated measurements of arterial pressure were taken on 3 healthy rabbits throughout the period during which clearance coefficients were being determined; observations of arterial pressure were carried out during each experiment on hypertensive rabbits. No significant alterations of blood pressure were discovered.

EXPERIMENTAL RESULTS

The first and fundamental series of experiments was devoted to a study of correlations between renal circulation and renal renin content in experimental neurogenic hypertension.

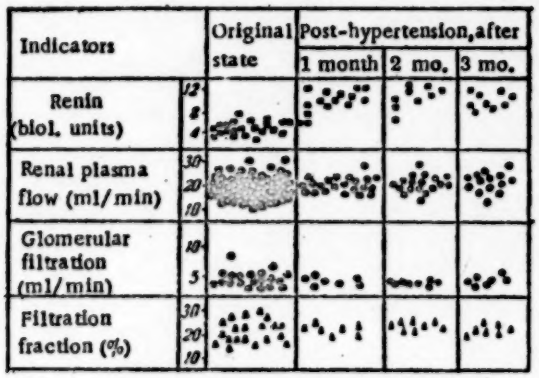

Fig. 1. Indicators of renal circulation and renal renin content at the onset of experimental "inhibitory" neurogenic hypertension.

Observations on indicators of renal functions were carried out over a period of 3 months following development of hypertension.

Renal blood flow was determined in 72 rabbits with normal pressures and in 22 of these it was again determined after onset of hypertension.

Glomerular filtration and, correspondingly, filtration fraction were determined in 20 healthy rabbits and traced in 8 of them during developed hypertension. Renal renin content was estimated in 22 rabbits with normal pressures and in 33 rabbits with experimental neurogenic hypertension.

As revealed by investigations, renal plasma flow (blood flow), glomerular filtration and filtration fraction in the normal showed very considerable differences (Fig. 1). Therefore, in order to evaluate these indicators after the development of hypertension it was necessary to determine the degree of their variability from day to day in one and the same normal animal. Results of 4 experiments staged for this purpose demonstrated pronounced individual fluctuations in the values which characterize renal function. Renal plasma flow and glomerular filtration in the same rabbit varied in different directions in the course of 1-1$^1/_2$ months up to 60-85% with respect to the original level. It was therefore considered to be preferable to compare the results obtained on hypertensive rabbits with all the data relating to the normal in order to achieve a more correct elucidation of changes in renal functions in hypertension.

The values for indicators of renal functions, as well as the wide range of their fluctuations obtained in the present investigation agree with data in the literature [13, 16, 18]. It seems significant also that the values for renal blood flow correspond to the results of direct blood flow determinations (Rein method) [21, 23].

During experimental neurogenic hypertension the values characterizing renal blood flow, glomerular filtration and filtration fraction did not leave the limits of the original figures (Fig. 1). Consequently no changes in renal circulation could be detected during 3 months following the onset of experimental neurogenic hypertension. At the same time, as can be seen from Fig. 1, the renin content of the kidneys showed marked changes during the same stages of the development of hypertension. As early as the first month there was considerable increase in renin content; this remained essentially constant in the following two months. Similar results were obtained by V. N. Chikvaidze [10].

Thus in the initial period of the development of neurogenic hypertension the increase in renal renin occurred in the absence of changes in renal circulation.

These data confirm the hypothesis postulated earlier by the present authors concerning the trophic nervous influences exerted on the kidney and maintaining the development of neurogenic hypertension.

R. Bing and later A. I. Vyshatina [12, 2] also did not observe any changes in renal blood flow and other renal functions in the first few months following development of experimental neurogenic hypertension. Vyshatina suggests that this indicates absence of renal pressor mechanism involvement in the pathogenesis of the early stages of neurogenic hypertension.

In the light of the results reported above this suggestion must be rejected as erroneous.

The second series of experiments consisted of a study of the effect of renal denervation on the correlation of renal circulation and renal renin content.

Indicators of excretory renal function were studied in 12 rabbits with normal blood pressure, and renin content in 22, both after denervation of the kidneys. Similar determinations were carried out on 14 hypertensive rabbits following renal denervation.

As in the first series of experiments the data obtained after denervation of the kidneys were compared with all the data characterizing the orginal state of renal function.

Denervation of the kidneys led to similar dynamics of renal function indicators and renin content in the normal and hypertensive rabbits (Fig. 2 and 3). In the first postoperative days, during which blood pressure and renin content of the kidneys remained at the original level, indicators of renal circulation showed a sharp drop.

It must be supposed that these changes depend on the marked stimulation of afferent and efferent renal nerves caused by their section and subsequent painting with phenol. True exclusion of the kidneys from the system of nervous influences supervened later. During this period a fall of blood pressure and renal renin content were observed, while indicators of renal circulation became stabilized at the original level.

Thus the drop in blood pressure caused by denervation of the kidneys and the almost complete disappearance of renin occur without appreciable changes in renal circulation.

The absence of any effect of severance of renal neural connections on the excretory function of normal kidneys has already been noted previously [1, 3, 23].

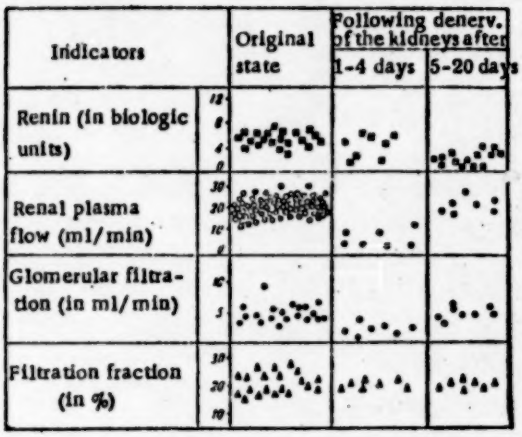

Fig. 2. Indicators of renal circulation and renin content following denervation of the kidneys in healthy rabbits.

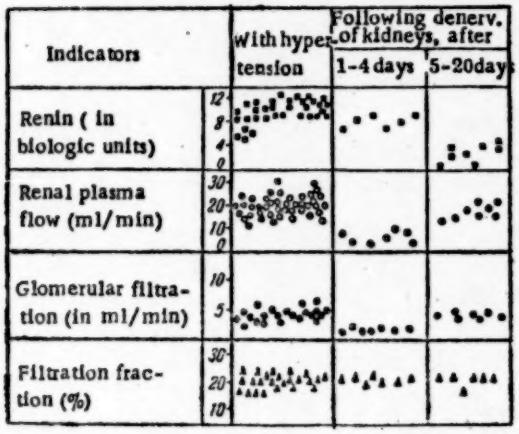

Fig. 3. Indicators of renal circulation and renin content following denervation of the kidneys in hypertensive rabbits.

It is important to remark in this connection that the workers [7, 14] who first discovered the depressor effect of denervation of the kidneys in experimental hypertension associated it with cessation of strong vasoconstricting influences. The present data refute such a hypothesis for the mechanism of cessation of hypertension following denervation of the kidneys. Exlusion of the renal pressor factor takes place independently from the state of renal circulation.

The results of the present work reject the significance of renal ischemia in the mechanism of neurogenic renal involvement, the renin system in particular, in the pathogenesis of experimental neurogenic hypertension, and thus confirm the point of view developed by V. N. Chernigovsky and his collaborators.

SUMMARY

Renin content of the kidneys and renal blood flow were determined in rabbits during the course of hypertension, induced by section of the pressor regulator nerves and after renal denervation, which abolishes neurogenic hypertension. The experiments revealed that trophic nervous influences on the kidneys, necessary for the induction of neurogenic hypertension, result in an excessive production of renin, which takes place in the absence of renal ischemia.

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CHANGES IN MYOCARDIAL REACTIVITY IN WHITE RATS TO REPEATED INJECTIONS OF ADRENALINE

O. P. Vishnevskaya

Hystophysiologic Laboratory (Head – Candidate of Biologic Sciences V. N. Dobrokhotov),
Institute of Experimental Biology (Director – Professor I. N. Maisky),
Academy of Medical Sciences USSR, Moscow

(Received August 23, 1956. Submitted by Active Member of the Academy of Medical Sciences USSR V. V. Zakusov)

It has been established that intramuscular injection of 0.5 or 0.8 ml adrenaline produces myocarditis in white rats. Frequent repetition of these doses with intervals no longer than 6 days do not result in repeated acute myocarditis, although there is shock with each injection. Rats sacrificed after many injections show only myocardial scarring similar to that seen in rats after a single injection sacrificed after the same period of time.

When frequent injections are given the animals tolerate doses of adrenaline which even exceed the lethal dose. This is evidently due to "areactivity" of the myocardium to repeated injections, since acute myocarditis is removed from the complex of reactions to adrenaline under these conditions [1].

Changes in myocardial reactivity were discovered morphologically. The effect of repeated injections on cardiac activity remained unclear. This question was studied by means of electrocardiography•.

Initially ECG changes following a single intramuscular injection of 0.5 ml adrenaline (12 rats) were determined. In the majority of the rats changes in the ECG appeared within 5 minutes after injection: displacement of the S-T_{CR4} interval above or below the baseline, diminution or inversion of T_{CR4} and increase or decrease in the voltage of R_{CR4}. These changes became more pronounced after 20-40 minutes, while after 24 hours they were present in all rats, in some cases to a very marked extent and not only in the chest lead IV but also in the three standard leads. Nine rats were sacrificed 24-25 hours after the injection. Microscopic examination of the heart disclosed acute myocarditis of varying intensity. The remaining rats showed the ECG changes described over a period of a week following the adrenaline injection. On the 7th day the S-T_{CR4} displacement was seen to be less marked, the voltage of R was restored and the T wave returned to normal in all leads. By the 12th day after injection the ECG was normal. The rats were sacrificed on the 23rd day after the beginning of the experiment.

Histologic examination of the hearts of these rats revealed only focal cardiosclerosis. The number and size of scars served as indicators of the severity of myocarditis.

These experiments showed that following injection of adrenaline, changes in the ECG often become apparent before the usual histologic methods can demonstrate the presence of inflamatory processes in the myocardium. Thus, 5-40 minutes after adrenaline injection the myocardium only shows hyperemia while the ECG points to disturbances of contraction and metabolic processes (reduction in voltage of the waves, S-T_{CR4} displacement and smoothing out or inversion of T_{CR4}.

Our previous experiments with alternating injections of adrenaline and sympatholitin led to the conclusion that the process underlying myocarditis began almost instantaneously following the injection of adrenaline. Morphologically, however, myocarditis is rarely apparent earlier than 3-6 hours after the injection. ECG also indicates the rapidity with which mechanisms underlying adrenaline-induced myocarditis are called into play – as

• The author acknowledges with gratitude N. A. Yushchenko's cooperation in this part of the work.

early as 5 minutes after the injection there are consistent changes in the ECG.

Towards the end of the first post-injection day both the histologic manifestations of myocarditis and the ECG changes are fully developed. Acute and subacute myocarditis lasts 6-8 days. During this time the ECG changes also persist. Scarring of the myocardium begins to take place from the 6th day. At the same time a beginning of ECG recovery can also be seen. By the 12th-23rd day, when the myocardial scars are fully developed, the ECG is little different from normal. Apparently focal cardiosclerosis does not always find reflection in the ECG.

Investigations have shown that ECG reflects the development of adrenaline-induced myocarditis, not only when it is morphologically evident but also in its very earliest, insidious period.

It was also found that there was correlation between the extent of ECG changes and the stage of development of myocarditis. In rats with diffuse and predominantly parenchymatous myocarditis the ECG changes were more profound and clear-cut not only in the chest lead IV but also in the three standard leads.

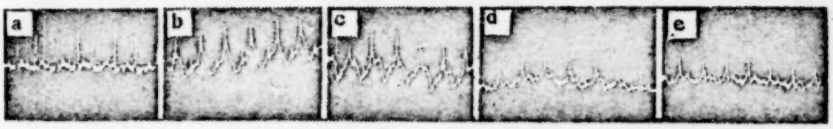

Fig. Rat No. 1029. Daily injections of 0.5 ml adrenaline. Rat sacrificed on the 9th day. Microscopic evidence of diffuse cardiosclerosis. Electrocardiograms – chest lead IV. a) 5/9/1955 ECG before the experiment. R = 0.7 mv; b) 5/10/1955. ECG 24 hours after first injection. R = 0.8 mv. S-T displacement above baseline; T negative; c) 5/12/1955 ECG 24 hours after third injection. R = 0.5-0.7 mv. T negative; d) 5/16/1955. ECG 24 hours after seventh injection. R = 0.5-0.8 mv. T weakly negative; e) 5/17/1955. ECG 24 hours after eighth injection. R = 0.5 mv. T positive.

In the next group of experiments (16 rats) injections of "myocarditic" doses of adrenaline (0.5 ml) were given daily. ECG records were taken before each injection and 40 minutes after it. The rats were sacrificed on the 9th day, 24 hours after the eight injection.

Forty minutes after the first adrenaline injection there was lowering or increase in the R_{CR_4} voltage and sometimes notching of this wave, displacement of the $S\text{-}T_{CR_4}$ interval above or below the baseline and lowering and sometimes inversion of T_{CR_4}. 24 hours after the first injection the same changes were seen, but more marked; in some rats they were seen in all four leads.

During the next 5-6 days no new changes were observed in the ECG.

Neither the first nor the subsequent injections were usually accompanied by disturbances of the cardiac rhythm.

Signs of recovery appeared in the electrocardiagram 6-8 days after the first injection: increased height or appearance of a positive T wave in all leads and restoration of the R wave voltage. On the 9th day the ECG of these rats differed little or not at all from normal (see Fig.).

Correlation of ECG changes with histologic data showed that the extent of ECG changes in this group of experiments corresponded to the severity of myocarditis associated with the first injection.

These investigations thus established that with daily injections of "myocarditic" doses of adrenaline only the first injection is significant with respect to the myocarditis. Further "areactivity" of the myocardium consists not only of the absence of new bursts of acute myocarditis following repeated injections, but also of the absence of repeated characteristic changes in the ECG.

There is no doubt that electrocardiographic changes reflect the appearance of acute myocarditis. They either remain on the same level or become somewhat more pronounced during the acute and subacute periods of myocarditis. As myocarditis changes to cardiosclerosis the ECG becomes more normal despite the daily

administration of "myocarditic" doses of adrenaline. Since it is taken that ECG reflects biochemical processes in the myocardium it would appear that the complex of reactions characteristic for adrenaline undergoes qualitative changes on repeated injections of large doses of this substance. There are references in the literature [2] to adaptation of the organism to adrenaline.

The data obtained point to adaptation to an "extraordinary" stimulus as being at the basis of the changed reactivity of myocardium to repeated large doses of adrenaline. This is more especially interesting since adrenaline under the particular experimental conditions is an "extraordinary" stimulus in the quantitative sense only, being a constant agent in the physiology of the organism. Such rapidly developing adaptation can be referred to the category of tachyphylaxis. This phenomenon, known to occur with respect to adrenaline as evidenced by functional indicators, can apparently also occur in such organic lesions as adrenaline-induced myocarditis.

SUMMARY

Frequent injections of large doses of adrenaline in rats, resulted in myocarditis with characteristic changes of ECG only after the first injection. As the myocarditis developed into cardiosclerosis, the ECG became normal in spite of daily injections of "myocarditic" doses of adrenaline.

The changed adrenaline response following the first injection may be regarded as an adaptive phenomenon.

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TOLERANCE TO COMBINED EFFECTS OF OXYGEN STARVATION AND TOXIC SUPPRESSION OF RESPIRATORY PHOSPHORYLATION AT DIFFERENT STAGES OF POSTNATAL DEVELOPMENT

S. V. Osipova

Chair of Pharmacology, Leningrad Pediatric Medical Institute

(Received May 1, 1956. Submitted by Active Member of the Academy of Medical Sciences USSR S. E. Severin

Disturbances of "macroergic" substance formation in the organism arise both in oxygen deficiency in the environment and in dissociation of respiration from phosphorylation by means of 2,4-dinitrophenol (DNP). The combined action of hypoxia and DNP enhances the harmful effect of both these factors on the organism [4].

Since the organism's tolerance to hypoxia is determined by the functional state of the central nervous system, and it in turn depends on the balance of "macroergic" compounds, decreased tolerance of animals to lowering of atmospheric pressure can serve as an indicator of the negative balance of such substances in the central nervous system and, in particular, as a test for poisoning with small doses of respiratory phosphorylation poisons.

A study was made of the correlation between the degree of impairment of respiratory phosphorylation caused by DNP and the organism's tolerance to deficiency of oxygen in the outside environment (i. e., degree of hypoxia).

EXPERIMENTAL METHODS

White mice of 4 age groups were used: 1) newborn and two days old, 2) two weeks old, 3) four weeks old and 4) sexually mature.

Oxygen starvation in these animals was induced by placing them in a pressure chamber – a glass bell 6 liters in capacity from which the air could be pumped out (using Komovsky's pump with electric motor attached). A mercury barometer connected to the rarefied interior of the chamber monitored the rate and constancy of decompression.

The experiments were performed under conditions of acute and short-lasting oxygen starvation: the rate of decompression in experiments with mature, four- and two-week old mice was two minutes (it was found that with slower decompression there was possibility of adaptation), and duration was 10 minutes. In experiments with newborn mice the rate of decompression was 5 minutes and duration 30 minutes.

In the majority of investigations devoted to the study of tolerance in animals at different stages of postnatal development to various types of oxygen starvation (including lowering of atmospheric pressure) no account is taken of the individual variations in tolerance and the need for sequential characteristics of tolerance of the different age groups. This led the present author to collection of her own data which are presented in Fig. 1.

Experiments with DNP were carried out on animals of the same age groups. The animals were given 0.01-0.2% solution of DNP subcutaneously with the addition of sodium bicarbonate 15-30 minutes prior to the beginning of decompression. The sub-lethal doses of DNP used did not cause any noticeable changes in the animals' behaviour (the minimal lethal dose for adult mouse is somewhat in excess of 25 μg per 1 g weight; the absolute

lethal dose is 40 μg/g).

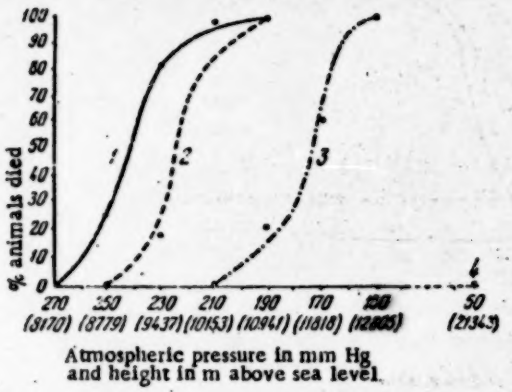

Fig. 1. Tolerance of white mice to lowering of atmospheric pressure at different stages of postnatal development:
1) Sexually mature animals (510 mice); 2) 4 weeks old (45 animals); 3) 2 weeks old (50 animals); 4) newborn (76 animals) (the newborn survive at pO_2 = 50 mm Hg).

Results of studies on the effect of DNP on the tolerance of mature mice to lowering of atmospheric pressure are given in Fig. 2.

EXPERIMENTAL RESULTS

The experiments showed that toxic suppression of respiratory phosphorylation caused a sharp drop in the tolerance of white mice to lowering of atmospheric pressure, or in other words, lowering of atmospheric pressure markedly diminished the tolerance of mice to toxic suppression of respiratory phosphorylation: the mice died from doses of DNP which were easily tolerated by them under ordinary conditions and could die at such levels of decompression which were relatively well tolerated by them without DNP.

Results of experiments on the effect of DNP on the tolerance of mice at early stages of postnatal development to lowering of atmospheric pressure are presented in the table.

Effect of DNP Injection on Tolerance of Mice to Lowering of Atmospheric Pressure

Age	Pressure in mm Hg	Control animals		Animals poisoned with DNP	
		Total Animals	Animals that died	Total Animals	Animals that died
4-week old	250	20	0	10	8
2-week old	210	20	0	10	9
Newborn	100	25	1	10	10
"	150	25	0	10	6
"	200	10	0	10	1

As the table shows, preliminary (30 minutes prior to decompression) administration of DNP (10μg, which is 50-70%MLD DNP for such age-groups) leads to fatal outcome at such lowering of atmospheric pressure which is not fatal for control (not poisoned) animals of corresponding age including newborn who are particularly

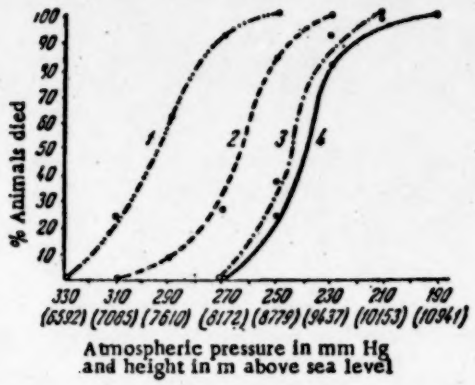

Fig. 2. Effect of various doses of 2,4-dinitrophenol (DNP) on the tolerance of mature mice to lowering of atmospheric pressure:
1, 2, 3) after DNP injection, 4) control; 1-20 μ g/g DNP (80 animals); 2) 5μ g/g DNP (136 animals); 3) 1μ g/g DNP (40 animals); 4) control (510 animals).

resistant to oxygen starvation.

Pronounced decrease of tolerance to lowering of atmospheric pressure on administration of DNP was also noted in experiments on frogs who became even less tolerant than normal newborn mice. These experiments were carried out on spring frogs whose oxygen requirement is higher than at any other season.

Experiments on frogs also demonstrated that preliminary administration of DNP caused a sharp decrease in the tolerance of the central nervous system to anoxia produced by ligation of the aorta: reflex turning over and reflex to mechanical stimulation of legs (pinching) disappeared several times more rapidly than in control frogs. In these experiments DNP was given in doses which did not affect the behavior of intact animals (5-10-20 μg/g of weight).

Experiments with preliminary (2-3 hours prior to deprivation of oxygen) administration of sodium fluoride to the four age groups of mice mentioned above in doses amounting to 50-70% MLD failed to confirm indications available in the literature [1, 3] that poisons of glycolytic phosphorylation lowered the tolerance of animals to oxygen starvation; in the present series of experiments little effect on the number of fatal cases was observed.

This divergence in results of the present work and some of the published investigations is explained by the fact that in some of the investigations lethal and even supra-lethal doses of the poisons were taken. The results of these investigations should be considered in the light of considerably greater effectiveness of respiratory phosphorylation as compared with glycolytic with respect to the formation of compounds called "macroergic" by V. A. Engelhardt.

SUMMARY

Toxic suppression of respiratory phosphorylation caused by 2,4-dinitrophenol, sharply decreased the resistance of white mice towards the reduction of atmospheric pressure. In adult mice this effect was caused by 1/5 MLD. During the first four weeks of life, the resistance of the animals was reduced by dinitrophenol doses comprising only 1/2-3/4 MLD.

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*In Russian.

CHANGES IN BLOOD SERUM PROTEIN FRACTIONS AS INDICATORS OF THE COURSE OF INFLAMMATORY PROCESSES

N. M. Rudoi

Laboratory of Pathologic Physiology (Chief – Professor G. E. Platonov), Tuberculosis Institute of the Academy of Medical Sciences USSR (Director Z. A. Lebedeva), Moscow

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The nature of the changes in protein fractions of blood serum as indicators of the course of inflammatory processes has not as yet been adequately studied.

Since there are references in the literature to the influence of inflammatory processes on the interrelations of serum protein fractions as revealed by electrophoretic separation [2, 3, 5, 6] this method was adopted for the present study of the proteinograms of those patients in whom a brief exudative inflammatory reaction could be expected to arise in connection with therapeutic measures. With this aim in view a survey was made of patients with pulmonary tuberculosis; these were subdivided into the following two groups, depending on the therapy received:

1. Patients who had been subjected to surgical intervention accompanied by inflammatory reaction of traumatic origin.

2. Patients treated with tuberculin which can cause focal reaction.

EXPERIMENTAL METHODS

The total amount of protein was determined by an immersion refractometer; the ratio of protein fractions by paper electrophoresis. Separation of the serum was carried out in the A. E. Gurvich [1] electrophoretic chamber over a period of 16 hours, on paper soaked in Veronal-Medinal buffer (pH 8.6, ionic strength 0.1) at constant current of 250 v and 0.1 mA per 1 cm of paper width. The paper strips were stained with bromophenol blue. Measurements of the electrophoregram were made in the specially constructed apparatus for direct photometry [4].

TABLE 1.

Percentage Content of Protein Fractions in the Blood Serum of Healthy Subjects

Globulins				Albumins
γ	β	α_2	α_1	
16.5 ±2.1	13.0 ±1.8	8.5 ±1.0	4.3 ±0.9	57.7 ±2.5

Studies on the blood serum protein fractions in the patients were preceded by similar studies on 20 healthy subjects. The following data were obtained as the result of these studies; calculations were performed by the variation method (Table 1).

These data served as a certain standard with which the changes in protein composition of blood serum in the patients could be compared.

EXPERIMENTAL RESULTS

The first group of patients was made up of 21 persons aged 16 to 40 years. They were subdivided according to the form of illness as follows: infiltrative pulmonary tuberculosis – 5 patients, focal – 3, chronic hematogenic-disseminated – 2 and chronic fibrous-cavernous – 11. Four patients had been subjected to thoracocauterization, 13 to extrapleural pneumolysis, one to conservative resection of the pulmonary lesion and three to lobectomy.

Blood for investigation was taken 3 times: prior to operation, on the 4th postoperative day and after disappearance of postoperative inflammatory changes. Development of unfavorable postoperative complications occurred in 2 patients. In one of them the last proteinogram determination was carried out shortly before death.

Table 2 reflects the dynamics of the protein composition of blood serum in all the patients under observation.

Data from 4 and 5 graphs enabled us to summarize the obtained material as follows (Table 3).

As Tables 2 and 3 show, in 17 patients there was a decrease in the amount of total protein as compared to preoperative values; in 3 patients it remained unchanged and in one there was an increase. In 16 of the 21 patients studied diminution of γ-globulin content occurred and only in five this fraction showed a slight rise. Concurrently with this there was an increase of total α- globulin in all patients, of α_2-globulin in 20 and α_1-globulin in 16. Concentration of albumin was decreased in all 21 patients. Changes in the β-globulin content varied: increases and decreases of this fraction were noted in an equal number of cases.

Thus the changes in protein values of blood serum in patients with pulmonary tuberculosis following operative intervention were characterized by a certain decrease in the concentration of total protein and of its γ-globulin and albumin fractions and an increase in α-globulins at the expense of α_2-globulin first of all.

Examination of the differences in the changes of the protein composition of serum associated with different operations revealed the following:

a) the least marked changes were seen after cauterization of pleural adhesions complicated by transient mild pneumopleurisy, and after segmental resection and lobectomy without postoperative complications. The average increase in the α_2-globulin level in this group of patients was 2.2% and the average reduction of albumins – 3.5%;

b) the most pronounced changes in the protein composition of the blood were seen in operated patients whose postoperative period following lobectomy was stormy and characterized by deterioration of the general condition, progressive course of the pulmonary process, development of empyema.

Clinical deterioration was accompanied by hypoproteinemia and a sharp fall in the albumin and γ-globulin levels with concurrent considerable increase in α-globulin content and moderate increase of β-globulin. The average increase in α_2-globulin in this group of patients was 6.6% and the average decrease in albumins – 9.4%.

c) the changes in the protein composition of the blood following extrapleural pneumolysis were of the same character but not so pronounced, occupying an intermediate position between the two degrees mentioned above. The average increase in α_2-globulin in this group was 4.2%, and the average reduction in albumins – 4.5%. Changes in protein fractions were accompanied by formation of exudate in the extrapleural cavity, serous-hemorrhagic at first, then serous. These inflammatory changes resolved favorably.

The data obtained showed that various operative procedures on the thorax caused changes in the protein composition of the blood whose degree depended on the presence and extent of postoperative exudative inflammation of the tissues. The persistence of such changes with respect to the individual serum fractions as well as subsequent dynamics of these changes usually reflected the intensity and duration of the inflammatory reaction in the postoperative period. As the postoperative inflammatory manifestations settled down the changes in the protein composition of the blood associated with them also disappeared: the amount of α-globulins decreased while that of albumins and γ-globulin increased.

TABLE 2

Dynamics of the Protein Composition of Blood Serum in Patients Treated Surgically (the total serum protein content is expressed in grams-per cent, protein fraction content – in percentage of total protein)

I. Operation – Cauterization of Pleural Adhesions

Exper. No.	No. of case history	Name	Prior to operative intervention						After operative intervention											
			Globulins				Albumins	Total protein	During development of pneumopleurisy						After complete resol. of pleural exudate					
									Globulins				Albumins	Total protein	Globulins				Albumins	Total protein
			γ	β	α_2	α_1			γ	β	α_2	α_1			γ	β	α_2	α_1		
1	2	3	4						5						6					
1	520	Kr-ba, E.M.	19.2	20.1	11.3	7.1	42.3	7.87	20.2	19.5	14.2	6.0	40.1	7.63	20.0	18.5	9.3	4.2	48.0	7.63
2	28	Od-va, V.L.	32.2	9.5	5.6	3.0	49.7	6.88	30.3	8.2	10.0	3.3	48.2	6.77	21.8	13.0	9.8	4.1	51.3	6.96
3	484	Si-aya, E.M.	24.1	13.9	12.9	4.9	44.2	6.77	22.9	10.4	13.3	9.5	43.9	7.20	25.6	6.1	11.6	8.4	48.0	7.46
4	416	Ga-ev, G.D.	19.5	12.5	9.6	5.4	52.6	7.73	20.0	14.5	13.2	7.4	44.9	7.46	25.0	9.9	10.6	4.3	50.2	7.87

II. Operation – Extrapleural Pneumolysis

			Prior to operative intervention						After operative intervention											
									During exudate formation						After complete exudate resolution					
1	457	An-va, R.N.	29.4	12.4	7.3	4.2	46.4	7.87	24.1	10.0	14.1	6.5	45.3	6.98	26.8	13.8	4.7	4.1	50.6	7.20
2	548	St-va, A.M.	20.1	13.8	10.5	10.3	45.3	8.1	18.1	15.5	16.9	6.3	43.2	7.98	20.7	13.2	9.5	8.6	48.0	7.98
3	469	Ma-ev, B.V.	15.9	15.3	10.9	5.8	52.1	7.44	17.4	11.7	15.6	6.5	48.8	7.55	17.7	15.7	9.0	5.0	52.6	7.22
4	439	Ze-an, M.S.	17.1	16.0	9.5	3.6	53.8	6.92	17.7	13.7	13.2	4.8	50.5	6.79	18.9	10.9	11.3	4.6	54.3	6.94
5	9	Mo-va, P.V.	23.2	19.3	5.0	4.0	48.5	7.42	12.6	13.4	10.4	11.5	43.1	6.85	18.7	15.9	9.6	6.6	49.2	7.46
6	539	Al-ev, M.M.	19.3	15.4	16.6	12.3	36.4	6.38	18.1	17.4	19.3	12.0	33.2	6.12	18.5	15.2	14.7	6.4	45.2	7.02
7	345	Ku-ish, M.S.	25.5	7.0	16.2	3.4	47.9	7.61	20.6	11.0	16.0	7.5	44.9	6.88	21.1	18.6	7.0	5.9	47.4	7.46
8	32	Ok-ev, A.I.	22.7	9.5	11.7	3.2	52.9	7.53	21.8	10.9	14.3	4.4	48.6	7.57	24.7	12.9	9.9	4.5	48.0	7.44
9	24	Se-ev, N.Ya.	25.7	11.9	6.3	3.2	52.9	7.85	24.3	5.9	16.1	3.7	50.0	7.42	20.5	11.9	10.6	5.7	51.3	8.06
10	23	Pa-in, N.S.	19.6	13.0	15.1	5.1	47.2	6.88	26.3	14.5	17.0	5.0	37.2	6.88	20.0	10.5	13.0	6.7	49.8	7.20
11	419	Fo-ev, N.F.	22.4	14.6	8.4	7.0	47.6	8.13	17.5	14.9	15.9	9.1	42.6	7.55	29.1	15.2	7.5	4.9	43.3	7.61
12	474	Li, Ch. S.	22.8	11.7	11.1	6.1	48.3	7.42	21.5	16.4	13.6	6.5	42.0	6.98	18.8	17.8	9.3	6.3	47.8	7.42
13	386	Zhi-na, M.I.	19.6	11.4	14.6	5.8	48.6	6.83	27.4	12.4	15.8	8.9	40.5	6.55	21.9	13.0	10.7	5.3	49.1	6.94

III. Operations Involving Resection of Pulmonary Tissues

			Prior to operative intervention						After operative intervention						No signs resol. congestive pleural exudate or partial resolution					
									During development of empyema											
1	452	Bu-ov, M.M.	26.9	13.1	13.2	6.2	40.6	7.44	22.3	15.0	20.5	10.8	31.4	6.34	20.2	17.1	23.4	11.7	27.6	6.12
2	348	Zi-li, A.P.	25.6	12.2	11.0	4.7	46.5	7.59	17.9	15.3	16.9	12.8	37.1	6.49	19.9	11.5	14.0	6.2	48.4	7.89
			Prior to operative intervention						In the absence of pleural reaction						During further smooth postoperative course					
3	450	Sa-in, D.I.	25.9	8.5	12.9	5.8	46.9	7.96	23.5	18.1	13.8	5.4	39.2	6.53	20.5	17.4	10.5	7.2	44.4	6.94
4	463	Ba-va, O.G.	24.4	11.8	13.1	2.9	47.8	7.63	22.8	11.0	14.5	5.7	46.0	7.22	23.4	12.7	10.8	1.7	51.4	8.06

TABLE 3

Nature of the Changes in Blood Serum Proteins Seen in Patients with Pulmonary Tuberculosis Following Operative Intervention

Nature of changes in proteins after operative intervention	Total protein	Fractions of globulins					Albumins
		γ	β	α_1	α_2	Total α	
	Number of patients						
Increase in protein indicators	1	5	10	20	16	21	—
Unchanged protein indicators	3	—	—	—	—	—	—
Decrease in protein indicators	17	16	11	1	4	—	21

The second group consisted of 10 patients aged 16 to 45 years. Five of these suffered from infiltrative, four from focal and one from chronic hematogenic-disseminated pulmonary tuberculosis. All 10 patients were being treated with tuberculin against the background of preceding and continuing streptomycin and PASA therapy. The addition of subcutaneous injections of tuberculin was determined by the wish to facilitate the penetration of the antibacterial preparations mentioned into the foci of tuberculous inflammation and to stimulate healing processes.

The blood proteins were determined 3 times in this group: on admission to the clinic, immediately before beginning tuberculin therapy following preliminary treatment with antibacterial preparations, and on completion of treatment. In those cases in which clinico-radiologic data following one of the tuberculin injections showed development of focal reaction additional determinations were carried out.

In two of the 10 patients the supplementary tuberculin therapy did not produce any appreciable improvement in the course of the disease. Correspondingly, the content of α-globulins remained high, that of albumins low and of γ-globulin almost unchanged in these patients.

TABLE 4

Dynamic Changes in the Protein Composition of Blood Serum
Patient R-g (Case History No. 460)

Proteinogram	Globulins					Albumins	Total protein
	γ	β	α_2	α_1	Total α		
I	23.4	10.0	15.3	3.5	18.8	47.8	6.64
II	19.5	12.9	14.1	4.2	18.3	49.3	7.20
III	15.6	14.3	16 3	5.4	21.7	48 4	7.20
IV	20.9	11.6	9.2	7.0	16 2	51.3	6 98
	Patient I-na (Case History No. 559)						
I	13.8	10.6	14.8	3 8	18.6	57.0	6.98
II	15.1	10.5	8.1	6.7	14.8	59.6	6.77
III	16.0	15.2	11.1	5.7	16.8	52.0	7.20
IV	16.9	15.8	9.6	3.7	13.3	54.0	6 80

In 6 patients the combination of tuberculin therapy with antibacterial preparations exerted a positive effect on the course of the tuberculous process. It assisted the resolution of infiltrative and focal lesions and in some patients the closure of necrotic cavitation in the pulmonary tissue. Definite changes in the protein composition of blood serum accompanied this: normalization of α-globulins content, increase in the concentration of albumins and increase in the γ-globulin content.

In 2 patients very marked changes in the ratio of the protein fractions were noted during treatment. They were associated with focal reaction caused by tuberculin. The latter took the form of perifocal infiltration around the main focal lesion. At this time there was an increase in the total α-globulins content, mainly the α_2-globulin. The γ-globulin decreased in one patient and remained almost unchanged in the other. After a brief interruption the tuberculin injections were resumed. The patients' general condition and the local process showed considerable improvement; concurrently there was lowering of total α-globulins and increase in blood serum albumins. The dynamic changes in the protein composition of blood serum in these two patients are presented in Table 4. Proteinogram I reflects the state of serum proteins on admission of these patients, II -- before adding tuberculin therapy to treatment with antibacterial preparations, III -- during development of focal reaction, produced by subcutaneous injection of tuberculin, and IV -- just before discharge from the clinic, with favorable outcome. The total serum protein content is expressed in grams-per cent, while protein fractions -- -- in percentage of total protein.

Changes in β-globulin did not show clear consistency in all the 10 patients; fluctuations in total protein did not leave the limits of normal.

Thus, inflammatory changes of an exudative nature in the lungs, pleura and extrapleural cavity proceed against the background of appreciable disturbances of the blood serum protein composition; the latter are characterized by increased content of α-globulins (mainly α_2-globulin) and decreased level of albumins.

Changes in the protein composition of blood serum, determined by electrophoretic fractionation, can serve, together with other methods of investigation, as one of the indicators of the phase of inflammatory processes.

SUMMARY

Paper electrophoresis was used in the determination of serum proteins in patients with various forms of tuberculosis. The dynamics of the exudative inflammation of the lungs, pleura and extrapleural cavity was characterized by a rise on α-globulins, mostly α_2-globulins of the blood serum and a decrease of the albumin level.

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THE MECHANISM OF PYRAMIDON-INDUCED CONVULSIVE SEIZURES

V. I. Zapadnyuk

Chair of Pathophysiology (Chief – Professor I. I. Fedorov),
Lvov Medical Institute (Director – Professor L. N. Kuzmenko)

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Academy of Medical Sciences USSR N. I. Grashchenkov)

Yu. I. Detsik and I. I. Fedorov [1] revealed the epileptogenic properties of Pyramidon in experiments on dogs, rabbits and cats, and put forward the suggestion that this preparation might be used in the treatment of schizophrenia. At present intravenous injection of Pyramidon in convulsive and subconvulsive doses has found clinical application in psychiatry for treatment of chronic forms of schizophrenia [4].

The aim in the present study of the convulsivant action of Pyramidon has been to elucidate the conditions on which the appearance of convulsive seizures depends.

Under novocaine analgesia the subcutaneous vein in the shin of a pup was exposed and a freshly prepared 4% solution of Pyramidon injected into it. After this the animal was closely watched.

Data presented in Table 1 show that intravenous administration of Pyramidon to pups aged up to 1 month did not result in convulsive seizures. Reaction to the injection of Pyramidon consisted of the following. Immediately following the injection, agitation, squealing and sometimes slight tremor were noted. Pups aged over 2 weeks exhibited in addition copious salivation; many were doubly incontinent and tonic extension of limbs was also seen; this resembled the tonic phase of a convulsive seizure. No epileptiform seizures were observed in pups under 1 month of age.

Pups over 1 month of age showed clear tonic contraction of all muscles but particularly in the extremities after administration of Pyramidon; increased salivation, severe panting and vomiting also became evident.

Typical epileptiform seizures following administration of Pyramidon were constantly observed in the case of pups older than 50 days and only in one experiment were they seen in a pup aged 30 days. The seizures were characterized by the presence of tonic and clonic phases and a period of automatic movements ("running on one spot").

Four pups aged 40 to 60 days were given Pyramidon in divided doses of 81, 124, 142, 165 mg/kg with no resultant seizures; as in pups aged under 1 month there was only tonic contraction of the muscles.

The epileptiform seizures in pups following administration of Pyramidon were characterized by the young animals often having not single but 2-3 seizures which sometimes passed into status epilepticus. Older pups were more sensitive to intravenous injection of Pyramidon. While doses of Pyramidon of 197-306 mg/kg were not lethal for pups under two weeks of age, doses of 90-165 mg/kg were lethal for pups aged $1^1/_2$-2 months.

In order to discover a relationship between the appearance of convulsive seizures and the rate of injection of Pyramidon 62 experiments on 16 dogs were carried out. Convulsive doses of Pyramidon were injected subcutaneously into the shin either slowly through a fine needle or with intervals of 0.5-2 minutes.

It must be remarked that while there are certain variations in doses of Pyramidon producing convulsions, for one and the same animal under similar conditions of administration the convulsive dose remains constant.

ABLE 1

ffect of Intravenous Injection of Different Doses of Pyramidon to Pups of Various Ages

o. of up	Sex	Age in days	Weight (in kg)	Dose of Pyramidon (in g/kg body wt.)	Animal's state after administration of Pyramidon	Outcome
1	Female	2	0.41	0.24	No convulsions	Survived
2	"	6	0.64	0.119	" "	"
3	"	40	2.9	0.165	" "	Died
2	Male	15	0.73	0.219	" "	Survived
2	"	43	3.51	0.081	" "	"
3	Female	15	1.2	0.127	" "	"
3	"	29	2.31	0.105	" "	"
3	"	68	4.17	0.114	Typical convulsive seizure	"
3	"	83	4.45	0.121•	" " "	—
4	Male	46	2.9	0.124	No convulsions	Died
5	"	56	7.8	0.048•	Typical convulsive seizure	Survived
6	Female	56	6.2	0.058	Typical convulsive seizure	"
6	"	74	5.6	0.085•	" " "	"
7	"	6	0.51	0.69	No convulsions	Died
8	"	6	0.48	0.227	" "	Survived
8	"	9	0.54	0.222	" "	"
8	"	30	2.9	0.063	Typical convulsive seizure	"
9	"	17	1.5	0.107	No convulsions	"
9	"	19	1.62	0.079	" "	"
10	"	2	0.3	0.226	" "	"
11	Male	2	0.32	0.375	" "	Died on the third day
12	"	2	0.3	0.73	" "	Died
13	Female	2	0.32	0.306	" "	Survived
14	Male	11	0.71	0.197	" "	"
15	"	11	0.81	0.247	" "	Died
16	Female	50	1.42	0.099	Typical convulsive seizure	Survived
17	"	50	1.4	0.086•	" " "	"
18	"	15	0.89	0.135	No convulsions	Died
19	"	60	2.8	0.142	" "	Survived
20	Male	60	2.2	0.09•	Typical convulsive seizure	Died
21	"	60	2	0.06	" " "	Survived

• Typical single convulsive seizures changed to status epilepticus.

In one case (experiment on the dog Damka) when the animal was agitated before the injection, a convulsive seizure occurred after a small dose of Pyramidon (25 mg/kg). The injection was repeated on the same dog 6 days later when it was placid. Convulsions took place after 42 mg/kg of Pyramidon had been given.

When Pyramidon is injected slowly the convulsive seizure develops after an appreciably larger amount of the substance than the epileptogenic dose usual for the given animal. Injected in divided doses, the epileptogenic dose becomes even larger. With this method of injection epileptiform seizures develop after amounts of Pyramidon 2-2½ times greater than that required to produce a seizure on rapid injection.

It was noticed that almost no toxic sequalae were observed in adult animals following repeated and frequent injections of Pyramidon. Half an hour after a convulsive seizure the animals behaved in a manner almost indistinguishable from the usual.

The development of convulsive seizures with smaller amounts of Pyramidon when it is injected rapidly evidently depends on the intensity of stimulation of receptors in the vascular bed. With rapid injection excitation of angioreceptors is greater since the solution of Pyramidon is diluted to a lesser extent than on slow injection.

In the next series of experiments Pyramidon was injected into the common carotid artery. With this method of administration more nearly optimal conditions are created for the action of Pyramidon on the carotid sinus area, vascular receptors of the brain and its meninges as well as directly on cerebral centers.

Experiments were performed on dogs and rabbits. 2 dogs (Burka and Rex) were given Pyramidon solution into the carotid artery externalized in a skin flap; in the other cases the common carotid artery was exposed under sterile conditions, 4% Pyramidon solution was injected into the artery and the wound closed. Results of these experiments are given in Table 2.

TABLE 2

Epileptogenic Doses of 4% Solution of Pyramidon on Injection into Common Carotid Artery

No. of Exp.	Description of animal	Weight in kg	Animal's	Artery receiving Pyramidon	Amt. Pyramidon (g/kg body wt.	State of the animal after injection
1	Bruka	15.6	4 years	Into right	0.0025	Convulsive seizure
2	Bruka	15.6	4 years	Into right	0.003	" "
3	Bruka	15.6	4 years	Into right	0.0043	" "
6	Bruka	15.6	4 years	Into right	0.0038	" "
12	Bruka	16.1	4 years	Into right	0.0039	" "
4	Rex	11.2	3 years	Into right	0.016	" "
5	Malyutka	7	5 years	Into right	0.0068	" "
7	Roza	17.7	4 years	Into right	0.0033	" "
8	Roza	17.7	4 years	Into left	0.0049	" "
9	Pup	5.6	2-5 months	Into right	0.0014	No convulsions
10	Female	5.6	2-5 months	Into left	0.085	Convulsions
11	Pup, male	6.1	2-5 months	Into left	0.013	No convulsions
13	Pup, male	6.1	2-5 months	Into left	0.062	Convulsions
14	Pushok	6.8	4 months	Into right	0.01	Convulsive seizure
15	Naida, male	3.2	4 months	Into right	0.093	" "
16	Zlaya	20.3	3 years	Into left	0.006	Convulsive seizure
17	Buran	16.5	3 years	Into right	0.031	" "
18	Lokhmach	20	2 years	Into left	0.07	No convulsions
19	Damka	15.6	4 years	Into left	0.005	Convulsive seizure
20	Damka	15.6	4 years	Into left	0.019	No convulsions
21	Damka	15.6	4 years	Into right	0.038	No convulsions
22	Bezymyanny	8	3 years	Into right	0.022	Convulsive seizure
23	Ryzhy	7.5	4 years	Into right	0.064	" "
24	Bely	7.8	3 years	Into right	0.082	" "
25	Gordy	11.5	4 years	Into left	0.142	3 convulsive seizures Dead from suffocation
26	Pup, 3 female	2.8	3 months	Into right	0.093	Convulsive seizure
27	Tsygan	20	5 years	Into right	0.1	3 convulsive seizures
28	Tsygan	20	5 years	Into left	0.008	Convulsive seizure
29	Ryabush	7	3 years	Into left	0.103	2 convulsive seizures
30	Zhuk	9.5	9 months	Into left	0.189	Status epilepticus
31	Pup, 4 female	2.6	3 months	Into right	0.015	Convulsive seizure
32	Aza	16.2	2 years	Into left	0.0056	" "
33	Aza	16.2	2 years	Into right	0.0066	" "

As Table 2 shows, the epileptogenic dose of Pyramidon on its injection into the carotid artery of adult female dogs was 2.5-6.8 mg/kg i. e., 10-20 times smaller than with rapid single intravenous injection. It is characteristic that convulsions in these cases took place instantaneously; they resembled the result of sudden electric current stimulation.

In the case of a 4-months old female dog (Pushok) a convulsive seizure appeared after injection of 10 mg/kg, i. e., a dose a little larger than that which evokes seizures in adult female dogs with the same method of administration. In 2 female pups aged $2^1/_2$-3 months a convulsive seizure was produced by injection of 85-93 mg/kg Pyramidon into the common carotid artery; the onset of convulsions was not immediate but 20-30 seconds after the injection, i. e., after a period similar to that usually observed after intravenous injections of Pyramidon.

Male dogs did not exhibit enhanced sensitivity to Pyramidon injection into the common carotid artery.

Females, on the other hand, reacted to intracarotid injection of Pyramidon with convulsive seizures with very small doses – 2.5-6 mg/kg. The epileptogenic dose of Pyramidon was, in the majority of cases of male dogs, little different whether administered by the common carotid route or intravenously although the experimental conditions were identical in the case of male and female animals.

The specific sensitivity to injection of Pyramidon into the common carotid artery in female animals and its absence in the males are not absolutely constant. Both increases and decreases of sensitivity (Tsigan, Experiment No. 28, Damka, Experiments No. 20-21 respectively) were observed at different times. It also depends on age; no increased sensitivity to Pyramidon injected by the common carotid route was, as a rule, observed in pups up to 3 months old.

In addition to the described experiments similar studies were made on rabbits and gave consistent data: convulsive seizures occurred on injection of Pyramidon into the common carotid artery in doses which were epileptogenic when given intravenously; this was observed both in male and female rabbits.

It was then shown that injection of Pyramidon into the femoral artery and external carotid artery in dogs produced convulsions when given in the same doses as intravenously. Suboccipital injection was followed by convulsions after 60-90 seconds when 10-15 mg/kg of Pyramidon had been administered.

The fact that convulsions following Pyramidon injection into the common carotid artery occur in many animals, predominantly female, with minute doses of the substance and with extremely rapid onset suggests that this method of administration may involve chiefly the receptor apparatus of the carotid sinus zone and possibly vascular receptors of the brain. Definite elucidation of this question requires further study.

Moreover, the direct action of Pyramidon on the central nervous system cannot be denied since this substance exerts considerable influence on basic cortical processes [2, 3].

SUMMARY

The effect of Pyramidon was experimentally studied to clarify the conditions of the rise of epileptic-like seizures.

It was noted that the development of a convulsive seizure in dogs when intravenously injected with a 4% Pyramidon solution proceeded differently depending upon the age of the animal, Pyramidon dose, rapidity and frequency of injection, and the site of the injection.

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BIOCHEMISTRY AND BIOPHYSICS

ABSORPTION OF RADIOACTIVE PHOSPHORUS FROM THE PLEURAL CAVITY

V. R. Faitelberg-Blank and Yu. B. Sosyura

Chair of Pathophysiology (Chief – Professor N. N. Zaiko), The N. I. Pirogov Odessa State Medical Institute (Director – Professor I. Ya. Deineka)

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There are a number of papers in the literature devoted to the question of absorption from serous cavities under various conditions.

Starling discovered the absorption of carmine and methylene blue from the pleural cavity in experiments on anesthetized dogs. Later it was found that other dyes as well as sodium chloride were readily absorbed from the pleural cavity and that this absorption was diminished when the central nervous system was excluded.

O. M. Goldina [1] observed the absorption of methylene blue from the pleural cavity in man. Studies on the absorption from the pleural cavity of various contrast media ("Sergosin" Thorotrast, barium sulphate) revealed that these substances were absorbed mainly through the parietal pleura [4]. Experiments on rabbits and mice were used for the study of penicillin absorption from the pleural cavity and showed that when the pleura was inflamed its absorption was reduced [6].

V. P. Plosky (1955) discovered that chloral hydrate and barbituric acid derivatives were well absorbed from the pleural cavity in animals. He succeeded in producing fairly prolonged narcosis in animals by intrapleural injection of narcotic substances.

The existing views on the spread of substances absorbed through the mesothelium can be summarized as follows: true solutions, once absorbed, pass predominantly into the blood capillaries and partially into lymphatic capillaries; colloidal solutions pass predominantly into the lymphatic vessels and partially into the blood vessels, while suspensions pass into the lymphatic system exclusively [3].

The mechanism of absorption from the pleural cavity is explained in various ways. While some authors explain absorption from serous cavities by physico-chemical factors (diffusion, osmosis), V. N. Orlov [5], A. A. Bogomolets (1908) and others regard it as an active biologic process regulated by the central nervous system.

The use of radioactive isotopes has made it possible to study the distribution of substances absorbed from the pleural cavity in the viscera (a study not previously feasible), using intrapleural injections of dye solutions or saline solutions.

The present work is concerned with the study of radioactive phosphorus absorption from the pleural cavity under ordinary conditions and against the background of artificial pneumothorax.

EXPERIMENTAL METHODS

Experiments were performed on 15 rabbits. Under manometric control radioactive phosphorus in the form of the sodium salt $Na_2HP^{32}O_4$ was injected into the pleural cavity in the dose of 50 million impulses per minute per 1 kg body weight. Samples of blood were then withdrawn from the pinna vein at constant intervals of time (3, 5, 10, 15, 20, 30, 45, 60, 90, 120 minutes) and their P^{32} content determined. Two hours after administration of P^{32} the animals were sacrificed (electric current) and the liver and lungs removed for P^{32} determination.

EXPERIMENTAL RESULTS

As a result of these experiments it was found that radioactive phosphorus was absorbed fairly rapidly and in appreciable amounts from the pleural cavity into the blood. Thus, 3 minutes after administration of radioactive phosphorus intrapleurally the percentage of its take up in the blood was 18.25 in rabbit No. 1, 18.75 in rabbit No. 2 and so on. The amount of radioactive phosphorus in the blood increased with time and in most experiments reached a maximum after 20-30 minutes (Table 1).

TABLE 1

Dynamics of Radioactive Phosphorus Absorption From the Pleural Cavity into the Blood

Experiment No.	Amount of phosphorus administered (imp/min/1 g tissue)	% uptake in blood after									
		3 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	90 min	120 min
Absorption under ordinary conditions											
1	5 100	18.25	33.4	41	47.3	56 9	63 1	61	83.2	90.7	108.3
2	5 112	18 75	22.9	43.75	84.75	94.30	69.7	40.75	83.5	64.25	101.1
3	5 225	—	20.45	47.9	56.5	72.1	93.5	63.73	60.0	50.75	99.1
4	5 200	18.5	44	37	54.5	59.1	86.3	100	80.4	67.5	96.4
5	5 250	23	57.2	92.5	130	144	150.2	129.1	123.1	119	124
6	4 436	42.5	35	68.5	131.6	66 5	62.1	59.5	89.5	79	73.7
7	5 254	80	79.3	80.1	90.1	91.3	84.2	105	85.5	74.1	86 7
8	5 000	80	29.4	47	89.2	71.5	58 2	101.2	97.2	100	100.3
Absorption under conditions of artificial pneumothorax											
9	4 900	14 7	34.6	45.7	69 38	62 6	134	65.7	61.8	41.25	50.4
10	5 010	24 4	36 6	45.5	61	51.1	47.1	82.5	55.5	36.7	49.5
11	5 200	16	29.9	52.7	74.25	107	133.2	—	122	129	59.2
12	5 085	21.8	33.2	48.3	68.2	69.5	121.6	74.1	71.5	52.3	46
13	5 200	18.3	38	51 8	62.2	71 6	127	68.3	50.4	32.8	40.5

M. Ya. Subbotin [7], studying absorption of dye-stuff from the abdominal cavity in rabbit, discovered that resorption occurred most vigorously during the first 30 minutes.

Investigation of the deposition of radioactive phosphorus absorbed from the pleural cavity in some of the viscera revealed that after 2 hours 1 g of liver contained such an amount of radioactive phosphorus that it gave, on the average, 10,048 imp/min with fluctuations from 5,604 to 16,056 imp/min. 1 g of left lung contained amounts of radioactive phosphorus giving the average of 7,069 imp/min with fluctuations from 2,800 to 16,880 imp/min. These data show that the radioactive phosphorus absorbed from the pleural cavity was deposited in the liver to a greater extent than in the lungs.

In the second series of experiments a study was made of radioactive phosphorus absorption from the pleural cavity in rabbit under conditions of artificial pneumothorax. The air was introduced into the right pleural cavity, under sterile conditions, thrice at 24 hour intervals by means of the Kochkachev pneumothorax apparatus.

Fifty ml of air was introduced in the first instance, 65 ml in the second and during the third administration the air was introduced until the intrapleural pressure reached slightly positive values (+ 2 cm of water).

The general condition of the rabbits after application of artificial pneumothorax was not altered appreciably. The increased rate of respiration noticed directly after the procedure returned to normal after a few minutes.

The results of these experiments demonstrated that the absorption of radioactive phosphorus from the pleural cavity into the blood in the presence of artificial pneumothorax was slowed down. Under these conditions the percentage uptake into the blood after 120 minutes was 49.5-59.2 (Table 1) as against 73.7-124 under normal conditions. It must be noted that, while the degree of absorption of radioactive phosphorus is lowered in the case of pneumothorax, its maximal accumulation in the blood takes place in the majority of cases similarly

to control animals, i. e., at the 30th minute. R. O. Faltelberg and L. B. Akselrod [8] observed slowing of neutral red absorption from the pleural cavity of dogs in the presence of artificial pneumothorax.

Deposition of radioactive phosphorus in the liver 2 hours after its introduction into the pleural cavity against the background of artificial pneumothorax occurred to a greater extent than in control animals (Table 2). It was also deposited to a greater extent in the left lung. Thus, under ordinary experimental conditions its percentage uptake in the left lung was 54-317.4 while with pneumothorax it was 368-552.5.

It must be mentioned that there is a difference between the deposition of radioactive phosphorus in the right and left lung under ordinary conditions and against the background of artificial pneumothorax. In the former case considerably more radioactive phosphorus is deposited in the right lung as compared with the left. This possibly depends on the fact that radioactive phosphorus is introduced into the right pleural cavity. In the case of artificial pneumothorax more phosphorus is deposited in the left, noncompressed, lung than in the right, compressed, lung.

When 1 g of right lung (on the side of pneumothorax) showed activity of 8,560-11,800 imp/min it reached 18,700-27,000 imp/min in the left lung (Table 2).

TABLE 2

Distribution of Radioactive Phosphorus Absorbed from the Pleural Cavity in the Viscera 2 Hours After its Introduction

Experiment No.	Hours after admin. of P	Amt. of P (imp/min/g tissue)	% blood uptake	Liver			Lungs (left)		
				no. imp/min/g liver	% uptake	activity of blood (in %)	no. imp/min/g lung	% uptake	activity of blood (in %)
Under ordinary conditions									
1	2	5 100	108	5 604	109	101	2 800	54	506
2	2	5 112	101	12 088	236	507,2	6 345	124.1	262
3	2	5 225	99.1	9 573	182	640	5 394	103	351
4	2	5 200	96.4	10 500	202	168	6 700	128 8	358
5	2	5 250	124	11 140	214	176	8 200	137	126
6	2	4 436	73.7	6 228	140	194	5 230	118	161
7	2	5 254	86.7	16 056	305	352	16 680	317.4	365
8	2	5 000	100.3	9 192	182,1	182	5 207	104.1	104
Against background of artificial pneumothorax									
9	2	4 900	50	10 400	213	417	27 000	552 5	1 100
							9 950	201	405
10	2	5 010	49.5	9 850	193	398	18 700	368	755
							11 800	236	480
11	2	5 200	59.2	16 000	306	525	21 000	403 8	688
							10 850	210	354
12	2	5 086	46	12 400	243,8	497	23 100	456	875
							8 560	167.5	322
13	2	5 250	40.5	11 650	221	394	20 400	388	685
							11 500	219	376

Note. In the column labelled "Lungs" in the section "Against background of artificial pneumothorax" the numerator values refer to data on the left lung, the denominators to data on the right lung.

No experimental data are available at present for the explanation of this phenomenon. It may be that the smaller deposition of radioactive phosphorus in the compressed lung is determined by impaired circulation in it. A big role is doubtless played also by neuro-reflex influences arising from pleural receptors in the course of artificial pneumothorax application. These questions are to be the subject of further investigations by the present authors.

The following conclusions can be drawn on the basis of the present experiments.

Radioactive phosphorus is rapidly absorbed from the pleural cavity: it is detected in the blood in appreciable quantities as early as 3 minutes following its introduction into the pleural cavity. Its accumulation reaches a maximum after 20-30 minutes, followed by a gradual decline; in a number of cases a second maximum is seen after 120 minutes.

The radioactive phosphorus absorbed from the pleural cavity is deposited in the viscera. As soon as 2 hours later radioactive phosphorus is found in considerable amounts in the liver and lung, its content in the former being higher than in the latter (left).

Radioactive phosphorus absorption from the pleural cavity proceeds less vigorously under conditions of artificial pneumothorax. It is first detected in the blood after 3 minutes and reaches a maximum at the 20th-30th minute, as in the case of ordinary conditions; after 2 hours the amount of radioactive phosphorus absorbed into the blood is considerably less than under ordinary conditions.

Radioactive phosphorus absorbed from the pleural cavity in the presence of artificial pneumothorax is deposited in the liver and lungs to a greater extent than under ordinary conditions.

More radioactive phosphorus is deposited in the left, uncompressed, lung as compared with the right one which is compressed by the air.

SUMMARY

Absorption of radioactive phosphorus from the pleural cavity was studied under normal conditions and against the background of artificial pneumothorax. Radioactive phosphorus in the form of a sodium salt was injected at the rate of 50 million impulses per minute per kg of body weight into the pleural cavity under manometric control. Deposition of radioactive phosphorus in the blood and in internal organs (liver, lungs) was determined. Three minutes after the injection radioactive phosphorus was evident in the blood, the maximum concentration being reached in 20-30 minutes. Two hours later, considerable portions of radioactive phosphorus were evident in the liver and lungs.

Artificial pneumothorax lowered absorption of radioactive phosphorus, but raised its deposition in the liver and lungs. Deposition of radioactive phosphorus in the left normal lung was higher than in the right collapsed one.

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β-RADIATION OF THE FETAL FLUID IN MAN

A. I. Danilenko

The A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR
(Director – Doctor of Medical Sciences Professor A. F. Makarchenko)

(Received December 31, 1955. Submitted by Active Member of the Academy of Medical Sciences USSR Professor L. V. Gromashevsky)

The question concerning the role of micro-doses of radioactive radiations as a biologic factor arose soon after the biologic action of macro-doses of radioactive radiations had been established.

As early as 1903 A. P. Sokolov [7] pointed out the connection between the therapeutic properties of natural climatic and balneologic factors and the presence of radioactive elements and increased atmospheric ionization.

The well-known experiment performed by Zvaardemaker on isolated frog heart demonstrated that the heart could pulsate for a long time only when the Ringer solution contained potassium ions.

Potassium is the only radioactive element which is concentrated in the organism of man and animals in amounts considerably in excess of its average content in the earth's crust. It possesses radioactivity which belongs to the isotope K^{40}, which constitutes 0.012% of the total amount of potassium.

Suggestions were made in a number of published works [5, 7] on potassium content of organs and tissues that the physiologic importance of potassium might be connected to a considerable degree with its radioactivity.

Micro-quantities of other radioactive elements besides potassium are contained in animal and plant organisms. In the case of man these gain access by way of food, water and air. Radiation from these micro-quantities of radioactive elements in the human organism constitutes less than 2% of potassium radiation [1].

Furthermore, animal and plant organisms contain artificially radioactive carbon isotope C^{14} formed as the result of cosmic ray action. It is present in amounts around 10^{-9}% of the total amount of carbon. To this should also be added the continuous action of cosmic radiation.

In recent years there have appeared in the literature views suggesting that animal and plant organisms were not indifferent to these radiations of very weak activity [3, 6].

Some investigators attempted to prove by direct experimental means that changes in the micro-doses of radioactive radiations caused changes in the development of organisms. They encountered great difficulties, however, since at the present time it is impossible to create conditions completely excluding penetrating radiations.

Another line of research lies along studies and accumulation of data concerning micro-radiations of radioactive elements contained in organisms. Correlation of data obtained under natural conditions can, albeit indirectly, lead to correct conclusions regarding the role of micro-doses of radioactive radiations.

One of the links in such a chain of investigations can be a study of radioactivity of fetal fluid in man.

S. P. Vinogradova [2] studied the biologic, physical and chemical properties of fetal fluid and G. P. Mushegyan [4] directed his attention to certain biologic aspects of this fluid. In 1927-1928 G. G. De-Metz tried to determine the radioactivity of human fetal fluid. However, he obtained negative data which may have been connected

with poor sensitivity of the method employed by him, and the question remained unresolved.

EXPERIMENTAL

The present work is devoted to studies of radioactivity of fetal fluid. Samples of fetal fluid obtained from several therapeutic establishments were subjected to counter examinations. The fluid used was in the form of light dispersed medium, uncontaminated by blood.

TABLE 1

Radiation Activity of Fluid in Which a Female Fetus Developed

Therapeutic establishment	Number of case history	Mineral residue 100 ml fluid in g	Specific activity of mineral residue in Cu units	Activity referred to 100 ml fluid in Cu units
Protection Maternity and Childhood	1756	0.625	$1.33 \cdot 10^{-10}$	$0.84 \cdot 10^{-10}$
October Hospital	1961	0.644	$1.28 \cdot 10^{-10}$	$0.82 \cdot 10^{-10}$
" "	2313	0.680	$1.07 \cdot 10^{-10}$	$0.73 \cdot 10^{-10}$
" "	2380	0.636	$1.00 \cdot 10^{-10}$	$0.64 \cdot 10^{-10}$
" "	2061	0.615	$1.02 \cdot 10^{-10}$	$0.63 \cdot 10^{-10}$
" "	57	0.621	$1.00 \cdot 10^{-10}$	$0.62 \cdot 10^{-10}$
Protection Maternity and Childhood	1760	0.722	$0.80 \cdot 10^{-10}$	$0.58 \cdot 10^{-10}$
October Hospital	2315	0.654	$0.88 \cdot 10^{-10}$	$0.57 \cdot 10^{-10}$
" "	2371	0.570	$1.00 \cdot 10^{-10}$	$0.57 \cdot 10^{-10}$
" "	2339	0.615	$0.93 \cdot 10^{-10}$	$0.57 \cdot 10^{-10}$
Protection Maternity and Childhood	1756	0.480	$1.16 \cdot 10^{-10}$	$0.56 \cdot 10^{-10}$
October Hospital	б/№	0.599	$0.88 \cdot 10^{-10}$	$0.53 \cdot 10^{-10}$
" "	2131	0.570	$0.90 \cdot 10^{-10}$	$0.51 \cdot 10^{-10}$
" "	351	0.668	$0.76 \cdot 10^{-10}$	$0.51 \cdot 10^{-10}$
" "	121	0.636	$0.74 \cdot 10^{-10}$	$0.47 \cdot 10^{-10}$
" "	302	0.570	$0.80 \cdot 10^{-10}$	$0.45 \cdot 10^{-10}$
" "	53	0.600	$0.74 \cdot 10^{-10}$	$0.45 \cdot 10^{-10}$
" "	223	0.588	$0.74 \cdot 10^{-10}$	$0.44 \cdot 10^{-10}$
" "	2404	0.577	$0.68 \cdot 10^{-10}$	$0.34 \cdot 10^{-10}$
Average			$0.93 \cdot 10^{-10}$ cu	$0.57 \cdot 10^{-10}$ cu

Direct measurement of radiation activity of fetal fluid is exceedingly difficult owing to its low intensity. Therefore, in order to obtain values characterizing radiation activity in curie units (cu) studies were carried out on ashed dry residues obtained by preliminary evaporation.

The measurements revealed that specific activity, i. e., activity referred to 1 g ahsed mineral residue, varied within the range of 0.20×10^{-10} to 1.33×10^{-10} cu.

Activity referred to 100 ml of fluid correspondingly varied within the range of 0.13×10^{-10} to 0.84×10^{-10} cu.

The activity measured in the present work was chiefly contributed by potassium as the apparatus used could record only beta-radiation with electron energy not less than 100,000 electron-volts (10^{-5} ev). Even

TABLE 2

Radiation Activity of Fluid in Which a Male Fetus Developed

Therapeutic establishment	Number of case history	Mineral residue in 100 ml fluid in g	Specific activity of mineral residue in Cu units	Activity referred to 100 ml fluid in Cu units
Protection of Maternity and Childhood October Hospital	1778	0.732	$0.63 \cdot 10^{-10}$	$0.46 \cdot 10^{-10}$
" "	224	0.733	$0.60 \cdot 10^{-10}$	$0.44 \cdot 10^{-10}$
" "	2376	0.655	$0.63 \cdot 10^{-10}$	$0.41 \cdot 10^{-10}$
" "	2400	0.643	$0.63 \cdot 10^{-10}$	$0.40 \cdot 10^{-10}$
" "	1983	0.680	$0.59 \cdot 10^{-10}$	$0.40 \cdot 10^{-10}$
" "	2241	0.729	$0.53 \cdot 10^{-10}$	$0.38 \cdot 10^{-10}$
" "	2243	0.607	$0.60 \cdot 10^{-10}$	$0.36 \cdot 10^{-10}$
" "	2378	0.680	$0.53 \cdot 10^{-10}$	$0.36 \cdot 10^{-10}$
" "	359	0.626	$0.45 \cdot 10^{-10}$	$0.33 \cdot 10^{-10}$
" "	74	0.605	$0.53 \cdot 10^{-10}$	$0.32 \cdot 10^{-10}$
" "	273	0.600	$0.53 \cdot 10^{-10}$	$0.31 \cdot 10^{-10}$
" "	184	0.550	$0.50 \cdot 10^{-10}$	$0.28 \cdot 10^{-10}$
" "	2132	0.550	$0.51 \cdot 10^{-10}$	$0.28 \cdot 10^{-10}$
" "	226	0.535	$0.50 \cdot 10^{-10}$	$0.27 \cdot 10^{-10}$
" "	б/№	0.636	$0.20 \cdot 10^{-10}$	$0.13 \cdot 10^{-10}$
Average			$0.53 \cdot 10^{-10}$ cu	$0.33 \cdot 10^{-10}$ cu

If other radioactive elements were present in the fluid their radiation must be so small that changes in activity caused by them would lie within the limits of experimental error which in these experiments did not exceed 10%.

It was found that radiation of fluid in which a female fetus had been developing (Table 1) was considerably higher than that of fluid in which a male fetus had been developing (Table 2).

SUMMARY

It was experimentally proved that the activity of β-radiation of the fetal fluid is different depending upon the sex of the embryo.

Thus, radiation activity of the fluid in which a fetus of the female sex (Table 1) was developing, was found to be significantly higher than that of the fluid in which a fetus of the male sex was developing (Table 2).

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MICROBIOLOGY AND IMMUNITY

PROTECTION OF THE CENTRAL NERVOUS SYSTEM FROM THE ACTION OF PERFRINGENS AND OEDEMATIENS IN RELATION TO THE TITER OF ANTITOXIN IN THE BLOOD

T. I. Bulatova and K. I. Matveev

N. F. Gamaleya Institute of Epidemiology and Microbiology AMN USSR (Director, Prof. S. N. Muromtsev)

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The necessity of an experimental study of the protection of the central nervous system by passive or active immunization against toxic infections became quite clear following the reports of a series of clinicians that with big doses of immune serum it was possible to cure serious cases of tetanus, diphtheria, gas gangrene, and botulism.

Up to that time, while the attending physicians used moderate doses of serum, the unsatisfactory results of serum therapy were ascribed to the fact that antibodies do not penetrate across the blood-brain barrier from the blood into the cerebro-spinal fluid [5, 3] and that the central nervous system is left unprotected by passive immunization (Roux and Borrel) [7, 5].

It was established by the majority of investigators that the penetration of various substances into the central nervous system depends, not on the composition of the fluid, but on the permeability of the capillaries and on the concentration of these substances in the blood [4]. In line with this, the penetration of antitoxins into the central nervous system, probably depends on the permeability of the capillaries of the brain and on the concentration of antitoxins in the blood and is completely unrelated to the presence of antibodies in the fluid.

The question arises, is the immune condition of the central nervous system altered by the intravenous injection of large doses of antitoxin, does it acquire in such cases a resistance to the action of toxins, in spite of the fact that antibodies practically do not get into the fluid?

EXPERIMENTAL METHODS

We carried out experiments with perfringens and oedematiens toxins on passively and actively immunized rabbits. We injected rabbits weighing 2.5-3 kg intravenously with various quantities of serum, then in about 4 hours, immediately after having taken blood and cerebrospinal fluid from them for determination of the titer of antitoxin, we injected the corresponding toxin into the cisterna magna.

The titer of the antiperfrigens serum was 850 AU/ cc, the titer of the anti-oedematiens serum was 100 AU/ cc. . The titration of the serum and cerebrospinal fluid was carried out by the usual methods employed in bacteriological institutes, intravenously in mice.

We carried out the experiments on the actively immunized mice according to the same program that we used for the passively immunized animals. Active immunization was carried out with the toxoids which we employed for the immunization of horses to produce therapeutic sera. We inoculated rabbits three times with 1-2 cc, with intervals of 20 days between the injections. About 12-15 days after the last inoculation we took blood and spinal fluid from them and injected them with the corresponding toxin.

For the experiments we used dried perfringens and oedematiens toxins obtained by precipitation with ammonium sulfate.

The minimum lethal dose (MLD) of perfringens toxin for mice injected intravenously was 0.3 mg, whereas the fatal dose for rabbits injected suboccipitally was 1.67 mg. The minimal lethal dose of oedematiens toxin for mice injected intravenously was 0.3 mg, the lethal dose for rabbits injected suboccipitally was 0.4 mg.

EXPERIMENTAL RESULTS

The results of the experiments with perfringens toxin in passively immunized rabbits are presented in Table 1. On the intravenous injection of the animals with large doses of antiperfringens serum, 1500-10,000 AU,

TABLE 1.

Effective Resistance of Passively Immunized Rabbits to Suboccipital Injection of Perfringens and Oedematiens Toxins

Name of Toxin	Experiment											Control		
	No. of Experiment	No. of Rabbits	Serum Injected (in AU)	Titer of Antitoxin (AU)		MLD Injected	Paralysis of Extremities		Died		Survived	No. of Rabbits	Toxin Injected	Died
				In Spinal Fluid	In the blood.		No. of Rabbits	On Day	No. of Rabbits	On Day				
Perfringens	1	2	10 000	>0.02 <0.1	—	10	2	2	2	3—4	—	—	—	—
	2	2	1 500	<0.02	>15<20	5	—	—	2	5--6 hours	—	—	—	—
	3	8	3 000	<0.02	>30<40	3	1	19	—	—	8	2	1	2
	4	5	1 500	<0.02	>15<20	3	3	3—7	3	6—8	2	1	1	1
	5	4	1 500	<0.02	=20	2	3	4—3	3	16—17	1	1	1	1
	6	5	1 500	<0.02	>15<20	1	3	13--15	—	—	5	1	1	1
Oedematiens	1	3	5 000	<0.02	>20<40	10	—	—	3	2	—	1	1	1
	2	5	5 000	<0.02	>20<40	5	—	—	—	—	5	1	1	1
	3	7	2 500	<0.02	>10<20	5	4	2—5	2	7—9	5	1	1	1
	4	4	2 500	<0.02	>10<15	3	—	—	—	—	4	1	1	1
	5	3	1 000	<0.02	>5 <10	3	3	2—3	3	4—7	—	1	1	1

and suboccipital injection of 5-10 MLD of toxin there was produced marked dyspnea, excitement; the rabbits ran around and screamed. The state of excitement lasted about $^1/_2$-1 hour. After some hours paralysis appeared in the animals and they died from the intoxication on the 2nd-4th day after the injection of the toxin.

We further injected rabbits with moderate doses of perfringens toxin (from 1 to 3 MLD), since these doses did not produce strong excitement of the animals. Only after a dose of serum equal to 3000 AU, following the injection of 3 MLD of toxin, did all the rabbits survive.

It is quite clearly evident that the larger the dose of antitoxin, the greater proportion of the animals are protected against the injection into them of a given amount of toxin: of 8 rabbits receiving 3000 AU, after the suboccipital injection of 3 MLD of toxin all survived, and only one showed paralysis of the hind legs on the 19th day, but out of 5 rabbits receiving 1500 AU, after the suboccipital injection of the same dose of toxin only two survived, while the others showed paralysis of the extremities and died. Out of 5 rabbits receiving 1500 AU and 1 MLD of toxin, all survived, though it must be admitted that three of them showed paralysis of the extremities on the 13-15th day. Untreated control rabbits always died on the 3rd-4th day following the suboccipital injection of 1 MLD of toxin.

On the injection of 5000 AU of antioedematiens serum (Table 1) and 10 MLD oedematiens toxin rabbits also exhibited significant excitement: the animals showed dyspnea and they ran around and screamed. Such a condition passed off rather quickly; after about 10-15 minutes the rabbits calmed down, and only the dyspnea remained. All 3 rabbits died on the following day.

From our experiments carried out on rabbits passively immunized with antioedematiens serum, it is quite clearly evident that the effective resistance of rabbits on suboccipital injection of toxin, and consequently the protection of the central nervous system both depend on the size of the dose of antitoxin injected.

Thus, for example, intravenous injection of a dose of serum containing 2500 AU completely protects the central nervous system of rabbits against the suboccipital injection of 3 MLD of oedematiens toxin whereas after the injection of serum containing 1000 AU the rabbits die following the injection of 3 MLD of toxin.

Intravenous injection of 5000 AU protects the central nervous system of rabbits against 5 MLD, since all 5 rabbits were unaffected by the suboccipital injection of this dose of toxin. Following the injection of serum containing 2500 AU not all of the rabbits survived the injection of 5 MLD of toxin. In our experiments, out of 7 rabbits 2 died, but it must be noted that 4 of the seven showed significant paralysis.

The titers of perfringens and oedematiens antitoxins in the blood of all passively immunized animals were sufficiently high, whereas under the conditions of our procedure no titratable amount of antitoxin was demonstrated in the cerebrospinal fluid.

Consequently the conclusion, reached by us in previous work [1, 2] with tetanus, botulinus and diphtheria toxins, that the protection of the central nervous system in passive immunization does not depend upon the penetration of antitoxins into the cerebrospinal fluid, remains correct for these cases.

The protection of the central nervous system depends on the titer of antitoxin in the blood. The higher the titer of antitoxins in the blood, the more antibody penetrates to the brain tissue through the capillary walls, while at the same time antibody is practically absent from the cerebrospinal fluid.

Our investigations explain the conclusions of many clinicians, based on practical observations during the treatment of gas gangrene, that the larger the dose of serum injected, the higher the therapeutic effect.

Shishkov [6] writes that the high titer of antigangrene serum of the post-war series, and the small quantity of protein ballast in it, offers the possibility of significantly increasing the dose of serum. With patients in peace time he employed for gas gangrene infections serum in the first two days in doses of 120,000 to 240,000 AU per day. Single doses of antigangrene serum employed in war time were from 40,000 to 60,000. Shishkov believes that the possibility of injecting antitoxin in a dose 3-6 times larger as compared to that used in wartime without doubt significantly increases the possibility of saving patients with gas gangrene infection.

A series of experiments were carried out by us on actively immunized rabbits. It is known that rabbits, which as the result of active immunization against perfringens or oedematiens have from 5 to 10 AU of antitoxin in their blood, can tolerate known lethal doses of a culture injected subcutaneously or intravenously.

However, up to now nobody has investigated how the central nervous system of actively immunized animals would react to the injection of toxin, introduced suboccipitally into the region of the brain.

In our experiments, out of 17 rabbits actively immunized with perfringens toxoid (Table 2) seven had an antitoxin titer $> 1 < 5$ AU, six rabbits, $> 1 < 3$, four rabbits $> 3 < 5$, and only one rabbit had 0.5 AU per cc of serum. Antitoxin was practically absent from the cerebrospinal fluid. Out of 12 rabbits receiving 1 MLD of toxin by suboccipital injection, 11 were completely unaffected and only one displayed on the 5th day signs of paralysis of the extremities; this animal died on the 9th day from the intoxication. Four control rabbits, receiving 1 MLD of toxin each, died on the 3rd-4th day. After the injection of 2 and more MLD of toxin into the immune rabbits, deaths began to be produced, since the degree of immunity was evidently not that high.

In the experiments with oedematiens toxin we obtained the same pattern. After immunization of 10 rabbits with toxoid a series of 127 blood antitoxin titers fell within the limits of 1 to 5 AU, and antitoxin was not present in the cerebrospinal fluid. Out of these 10 rabbits (Table 2) 3 rabbits receiving 1 MLD each of toxin suboccipitally were unaffected; out of 3 rabbits receiving 2 MLD each one died from the intoxication with signs of paralysis of the extremities. Out of 4 rabbits each receiving 3 or 5 MLD, only one survived.

In the second series of experiments (Nos. 5, 6, 7) 10 rabbits were immunized with toxoid of series 129. The titer of antitoxin in the blood was within the limits of 5 to 20 AU, and antitoxin was absent from the cerebrospinal fluid. All 6 rabbits which were injected with 2 or 5 MLD oedematiens toxin survived; it is true, however, that those which received 5 MLD each showed signs of paralysis of the hind legs. Out of 4 rabbits, each of which was injected with 10 MLD of toxin, only one was unaffected. After the injection of the toxin there was observed

TABLE 2

Effective Resistance of Actiyely Immunized Rabbits on Suboccipital Injection of Perfringens and Oedematiens Toxins.

Type of Toxin	Experiment									Control		
	No. of Experiment	No. of Rabbits	Titer of Antitoxin (AU)		MLD Toxin Injected	Paralysis of Extremities		Died	Survived	No. of Rabbits	Toxin Injected	Died
			In the cerebrospinal fluid	In the blood		No. of Rabbits	On Day					
Perfringens	1	12	<0.02	>0.5<5	1	1	5	1	11	2	1	2
	2	3	<0.02	>1<5	2	3	2—3	3	—	1	1	1
	3	2	<0.02	>1<5	5	2	—	2	—	1	1	1
Oedematiens	1	3	<0.02	> 1< 5	1	—	—	—	3	1	1	1
	2	3	<0.02	> 1< 5	2	1	4	1	2	1	1	1
	3	2	< 0.02	> 1< 5	3	1	3	1	1	—	—	—
	4	2	<0.02	> 1< 5	5	2	2—3	2	—	1	1	1
	5	2	<0.02	>10<15	2	—	—	—	2	—	—	—
	6	4	<0.02	>10<15	5	4	6—10	—	4	2	1	2
	7	4	<0.02	>10<15 —20	10	4	2—5	3	1	—	—	—

marked dyspnea and excitement, the animals screamed and threshed about. The antitoxin titer in the unaffected animal was 20 AU. In our experiments control untreated rabbits into which we injected 1 MLD of toxin each, always died on the 3rd-4th day.

Therefore, the protection of the central nervous system of actively immunized animals depends on the antitoxin titer in the blood. On the injection into the central nervous system of known lethal doses of toxins, only rabbits with a high titer of antitoxin in the blood survive.

SUMMARY

Experiments on passively and actively immunized rabbits with perfringens and oedematiens toxins showed that only the animals with a high blood antitoxin titer survive the introduction of lethal doses into the central nervous system.

These investigations throw light on the conclusion, reached by certain clinicians on the basis of practical experience in treating gas gangrene that large doses of serum produce better therapeutic results.

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ONCOLOGY

NEW STRAIN OF TRANSPLANTABLE MOUSE LEUKEMIA

Yu. M. Vasilyev

From the Laboratory of Neoplastic Strains (Chairman – Doctor of Biological Sciences E. E. Pogosyants) of the Institute of Experimental Pathology and Therapy of Cancer (Director – Corresponding Member of the Academy of Medical Sciences of the USSR Prof. N. N. Blokhin) of the Academy of Medical Sciences of the USSR, Moscow

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In recent years, the interest of Soviet researchers in the problem of the etiology, pathogenesis and therapy f leukemias and, especially, in the experimental study of this disease [3, 5] has increased noticeably. The ost convenient model for the study of some aspects of this problem, especially for experimental-therapeutic esearch, are the transplantable leukemias of mice. At present, only one strain of transplantable leukemia is eing sustained and used in the laboratories of our country – the strain LIO-I, obtained and described by L. L. alyugina [4]. Yet in carrying out experimental therapeutic research, comparative study of the action of this r that preparation on various strains of leukemia, which differ from each other in their properties, is important. n the basis of these considerations, we carried out a number of experiments whose purpose was to obtain a new rain of transplantable mouse leukemia.

In order to obtain a strain, we selected several mice of the afb variety with spontaneous leukemias and arried out hematological investigations. Test transplants were made of the leukemic tissue of these mice enlarged lymph nodes and thymus) to young mice of the same variety. The leukemia which caused the sharpest eukemic changes in the blood of the original mouse and in the first generation after transplantation was selected for further work. The blood of the original mouse contained 90,000 leukocytes per 1 mm^3, its differential lood count was: lymphoblasts 54%, prolymphocytes 18%, lymphocytes 8%, polymorphonuclear neutrophils 19.5%, osinophils 0.5%, monocytes 3%. The spontaneous leukemia we chose was fourth in number of those used in the ests, so our new strain was given the code designation LIV.

Later we carried this strain, by intraperitoneal (more rarely subcutaneous) transplants of a suspension of eukemic tissue, to mice of the variety afb for 1 – 1½ months. Before January 1, 1955, the strain was transplanted in 52 generations. With intraperitoneal transplant, local tumors did not appear in the mice, but generalized eukemia developed immediately, characterized by the same clinical symptoms as spontaneous mouse leukemia: general lassitude, more rapid respiration and enlargement of the peripheral (inguinal and cervical) lymph nodes. Usually the mice died no later than 1-3 days after the appearance of these symptoms. Small tumors at the place of transplantation appeared 7-10 days after subcutaneous transplantation of the leukemic tissue. However, these tumors did not have time to grow to a large size since generalization occurred in a few days and leukemia was found. It is interesting to note that the time in which the mice died of leukemia gradually decreased as the strain was transplanted (see Table). This increased speed of development of the leukemia, apparently, indicates that as it was transplanted, the strain gradually became more malignant. Such an increase in malignancy was observed repeatedly when various strains of malignant tumors were obtained [6]. Transplantable leukemias are no exception in this respect.

Usually hematological investigations of the blood were carried out on mice with already marked clinical symptoms of leukemia. The number of leukocytes in the blood of all the investigated mice was increased, very

considerably in the majority of cases. Thus, of 30 experimental animals, 7 were found to have from 30,000 to 50,000 leukocytes per 1 mm^3, 10 from 50,000 to 100,000, 6 from 100,000 to 200,000 and 7 over 200,000 per 1 mm^3.

Changes in the Time of Death of Mice When Strain LIV is Transplanted

Time death of mice (in days)	Generation		
	Minimum	Maximum	Average
1—10	10	30	18.3±1.0
11—20	9	24	14.4±0.4
21—30	7	20	12.8±0.3
31—40	7	15	10.3±0.3
41—50	7	12	9.5±0.2

The differential blood count of all the mice disclosed immature cells of the lymphocytic series – lymphoblasts, which formed from 22-75% of all the leukocytes. More mature lymphatic cells, prolymphocytes and lymphocytes, composed 20-40% of all the leukocytes in the majority of cases. In some animals, polymorphonuclear neutrophils composed only a small part of the leukocytes (from 10-20%), but in approximately half of the leukemic mice investigated the percentage of these cells was increased to 30-50%. If the considerable increase in the total number of leukocytes in these mice is taken into account, and corresponding recalculations are made, then it is found that the absolute number of polymorphonuclear neutrophils in these animals was increased (to 40,000-50,000 per 1 mm^3) in comparison with the normal (from 2600 to 15,200 according to the data of Ginzburg [2]). At the same time, mature forms of neutrophilic leukocytes always predominated in the blood; the percentage of young neutrophils was small (from 0 to 3).

Fig. 1. Leukemic infiltration of the spleen, enlarged 400 X.

Thus, the blood changes were characteristic of the lymphatic type of leukemia. Along with this, an increase in the number of neutrophilic leukocytes in the blood was noted in part of the animals. It is known [7] that such a leukemoid reaction is occasionally observed in mice when lymphatic leukemia is developing. Undifferentiated cells of the lymphatic type also predominated in smears of bone marrow.

Autopsy of mice which died of leukemia disclosed considerable enlargement of all the lymphatic nodes (inguinal, cervical, mesenteric etc.), the thyroid gland, the spleen and the liver, as well as some enlargement of both kidneys. On histological examination it was found that the normal tissue of the spleen, lymph nodes

and thyroid gland was almost completely replaced by leukemic tissue, consisting of fairly large atypical cells of rounded or polygonal shape, with a thin layer of cytoplasm and large, rounded, lightly-staining nuclei containing one or several large nucleoli (Fig. 1). Remains of lymph follicles were found only occasionally in this tissue. Numerous growth foci of the same leukemic tissue were found in the liver, located perivascularly, close to the central veins of the hepatic lobes. In addition, individual leukemic cells and small groups of such cells were located between the liver ligaments (Fig. 2). Leukemic infiltration was also found in the kidneys, located between the renal canals and below the capsule (Fig. 3). Large infiltrations were occasionally located

Fig. 2. Leukemic infiltration of the liver, enlarged 400 X.

Fig. 3. Leukemic infiltration of the kidney, enlarged 100 X.

in the perirenal fat. There was no leukemic infiltration of the lungs and cardiac muscle. We did not observe significant changes in the hematological and pathologic-anatomic picture of leukemia during transplantation of the new strain.

Thus, rapid development of generalized leukemic invasion of all the lymph nodes, liver, spleen and kidneys as well as the sharp changes in the blood picture and bone marrow typical of lymphatic leukemia, are characteristic of the strain described above. Bacteriological control of seedings from the organs of the dead animals gave negative results. The fact that transplantation of leukemia requires a transfer of cells is also confirmed by the variety specificity of the strain; all attempts to transplant leukemia LIV to mice of other varieties (C57, CC57 etc.) gave negative results. Strain LIV differs somewhat in its properties from Strain LIO-I. The appearance of a large leukemic tumor at the place of transplantation is characteristic of the latter, while leukemic invasion of the organs and leukemic changes in the blood do not develop in all the mice and are not always sharply evidenced. It is probable that experiments with strain LIO-I and with strain LIV can complement each other in experimental therapeutic investigations; the first strain is more convenient to study the effect of a given substance on a local leukemic tumor, while the effect of the same preparations on generalized leukemic invasion can be studied in experiments with strain LIV, and changes in the blood picture can be one of the criteria of the effectiveness of the preparations.

SUMMARY

Investigation of a new strain of transplantable lymphatic leukemia (LIO-I) in mice of the afb variety was carried out. The original leukemia arose spontaneously in the afb mice. After transplantation of LIO-I, leukemia in the form of a small local tumor appeared. In a few days, severe leukemic infiltration of lymphatic glands and internal organs developed. The number of leukocytes in the blood increased to 150,000-250,000.

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ON THE PROBLEM OF THE EXOGENOUS ORIGIN OF THE TUMOR ANTIGEN OF SARCOMA M-1

Z. I. Rovnova

From the Institute of Virology (Director – Professor P. N. Kosyakov) of the Academy of Medical Sciences of the USSR, Moscow

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The origin of specific antigenic substances in tumors, which are not transferred by filtrates, has not been discovered. It is not known whether these substances originate endogenously or whether, as many investigators suppose, they are substances of exogenic origin, i.e., originating exogenously to the organism.

Since one of the signs of the exogenic origin of the antigen is its ability to cause an immunological reaction in the organism in response to its parenteral administration, it would be possible to prove the exogenous origin of neoplastic antigen by discovering specific antineoplastic antibodies in the organism during the development of the neoplasm.

Many authors [1, 4, 6, 7, 8, 9, 11, 12, 13] found antibodies in animals with experimental neoplasms, which were not transferred by filtrates. However, in the majority of works, the complement fixation reaction was positive not only with neoplastic antigen, but also with the antigens from normal organs. In addition, investigation of the sera of healthy animals showed that they occasionally give a positive complement fixation reaction with neoplastic antigens. Therefore, the problem of the specifity of the antibodies which were found has also not been clarified.

The attempts of other investigators [2, 10] to discover complement-fixing antibodies in animals with developing neoplasms proved unsuccessful.

The aim of the present work was the comparative study of the sera of rats before infection with sarcoma M-1 and at various lengths of time after infection in order to discover the specific immunological changes occurring during the development of the neoplasm.

EXPERIMENTAL METHOD

Blood for the investigation was taken from the tail of the rats, by notching it and sucking the blood with vacuum, or from their heart before infection with the neoplasm and at various lengths of time after infection.

The serum which was obtained was heated for 30 minutes at 56° and kept in soldered ampules in the refrigerator. The sera of a single rat, taken at different periods, were tested in a single experiment in dilutions of 1:10, 1:20, 1:40, 1:80 by the complement fixation reaction.

The antigens were prepared from tissues of sarcoma M-1, liver and spleen of the rats by the usual method with physiological solution [5].

Before testing the rat sera for the presence of antibodies, the working dose and the specifity of the antigens were tested with the specific immune sera of rabbits (antineoplastic, antihepatic and antisplenic sera).

The rat sera, taken various lengths of time after infection with sarcoma M-1 (on the 12th, 14th, 16th, 18th, 20th, 24th, and 28th days), were tested by the complement fixation reaction by two parallel methods: the classical and the long cold method. In both cases the volume was 2.5 cc.

EXPERIMENTAL RESULTS

When the sera were investigated, the complement fixation reaction by the prolonged cold method gave a considerably greater number of sera than when testing was by the classical method*.

TABLE 1

Investigation of the Complement Fixation Reaction by Various Methods of the Sera of Rats after Infection with Sarcoma M-1

Method of investigation	Number of sera investig.	Complement fixation reaction		
		positive	negative	anticomplementary properties
Classical (at 37°)	38	2	32	4
Long (at 4°C)	46	20	22	4

Of 20 sera which reacted positively, 4 sera gave complement fixation best with rat liver antigen; 11 sera fixed the complement equally well with all the antigens investigated (M-1, liver, spleen) and 5 sera reacted predominalty with neoplastic antigen, reacting more weakly with other antigens.

Can it be assumed that the positive reaction which was obtained in our investigations occurs as a result of the appearance of antibodies in the rats during the development of the neoplasm?

In order to clarify this problem, the complement fixing properties of 39 healthy rat sera were studied. Of them, 11 sera gave a positive complement fixation reaction. The nature of the positive reaction was the same as when the sera of rats infected with the neoplasm were studied. Five sera fixed complement in the same way in the presence of all antigens, 6 sera reacted primarily with splenic neoplastic antigens. Therefore it cannot be assumed that the complement fixation which we discovered occurs due to specific neoplastic antibodies which appear in the rat's organism during the development of the neoplasm.

In order to discover whether immunological changes occur in the serum of rats during the development of sarcoma M-1, we studied the sera of rats before infection with the neoplasm, then at various lengths of time after infection.

The sera of 45 healthy rats gave a positive complement fixation reaction with rat antigens in 26% of the cases (12 sera), while 4 sera reacted equally with neoplastic, liver, and spleen antigens, while 8 sera reacted with neoplastic antigen more evidently than with the antigens of normal organs.

The investigation of the sera of the same rats (44 rats) on the 14-17th day after infection with the neoplasm showed some increase in the percentage of positive reactions. The nature of the positive reaction was the same as that of normal sera.

Of the sera taken from the same rats (39 rats) on the 25-28th day after infection with the neoplasm, 12 sera (30%) reacted positively: 7 sera were taken from rats with well-developed neoplasms and 5 sera from rats in which the neoplasms did not develop or, having developed, underwent reabsorption (3 sera reacted in the same way with all three antigens. One gave the strongest reaction with liver antigen, eight, while reacting with all the antigens, gave best fixation with neoplastic or splenic antigens). The sera of 25 rats gave a negative reaction. Of them, neoplasms were well developed in 21 (weighing from 13 to 85 g) and 4 rats had no tumors.

* The reaction of a delay in hemolysis of 1 plus and higher when the serum was diluted 1:10 was considered a positive complement fixation reaction.

TABLE 2

Complement Fixation Reaction (in a Cold Atmosphere) of Rat Sera Before Infection and After Infection With Sarcoma M-1 With Rat Antigens

Serum dilution	Before infection, antigens: M-1	liver	spleen	Control serum	On the 14-17th day, antigens: M-1	liver	spleen	Control serum	On the 25-28th day, antigens: M-1	liver	spleen	Control serum	Notes
10	+	+(+)	+	±	++++	++++	++++	±	+++	+++	+++	—	Neoplasm in ovary
20	±	±	±	±	++++	++++	++++	—	++	++	++	—	
40	—	—	—	—	++++	++++	++++	—	±	±	±	—	
80	—	—	—	—	++++	++++	++++	—	—	—	—	—	
10	±	±	±	±	—	—	—	—	++++	+++	+++(+)	+	Subcutaneous Tumor
20	±	±	±	±	—	—	—	—	++++	++	+++(+)	±	
40	±	±	±	±	—	—	—	—	+++	+(+)	++	—	
80	±	±	±	±	—	—	—	—	+++(+)	±	±	—	
10	+(+)	+(+)	+	+	—	—	—	—	++++	++++	++++	++++	The same
20	+(+)	+	±	±	—	—	—	—	++++	++++	++++	++(+)	
40	+	+	±	±	—	—	—	—	++++	++++	++++	±	
80	+	+	±	±	—	—	—	—	+++	+(+)	+(+)	±	
10	—	—	—	—	—	—	—	—	—	—	—	—	The same
20	—	—	—	—	—	—	—	—	—	—	—	—	
40	—	—	—	—	—	—	—	—	—	—	—	—	
80	—	—	—	—	—	—	—	—	—	—	—	—	
10	+++(+)	+++(+)	+++(+)	+++	+	+	+	—	—	—	—	—	The same
20	++	+(+)	+(+)	+(+)	+	+	+	—	—	—	—	—	
40	++	±	±	±	+	±	±	—	—	—	—	—	
80	±	—	—	—	+	—	—	—	—	—	—	—	
10	+++(+)	++	++	+(+)	++	+	±	±	—	—	—	—	The same
20	++(+)	+	+	±	++	+	—	—	—	—	—	—	
40	+(+)	+	±	±	±	±	—	—	—	—	—	—	
80	+(+)	±	±	—	±	—	—	—	—	—	—	—	

TABLE 3

Investigation of the Sera of Neoplastic RSK Rats at a Cold Temperature Before and after Absorption

Rat serum	Dilution of serum	Before absorption, antigens: M-1	Before absorption, antigens: liver	Before absorption, antigens: spleen	Before absorption, antigens: Control serum	Absorbed by	After absorption, antigens: M-1	After absorption, antigens: liver	After absorption, antigens: spleen	After absorption, antigens: Control serum
Rat No. 2 Ovarian tumor on 14th day after infection	10	++++	++++	++++	++	Formalin-treated splenic tissue, + alcohol-treated liver tissue	++(+)	++(+)	++	++
	20	++++	++++	++++	-		++	+	++	-
	40	++++	++++	++++	-		+	-	-	-
	80	++++	++++	++++	-		-	-	-	-
The same on the 25th day after infection	10	++	++	++	-	Formalin-treated splenic tissue	++	++	+(+)	-
	20	++	++	++	-		++	++	+	-
	40	++	++	+	-		+	+	-	-
	80	+	-	-	-		-	-	-	-
Rat No. 6 Ovarian tumor on 14th day after infection	10	++++	+++	-	-	Alcohol-treated liver tissue	++	++++	-	-
	20	++++	++++	-	-		++	++++	-	-
	40	++++	++++	-	-		(+)	++	-	-
	80	++	++	-	-		-	-	-	-
The same on the 25th day after infection	10	++++	++	-	-	Formalin-treated splenic tissue, + alcohol-treated liver tissue	++	+	-	-
	20	++++	++++	-	-		+	-	-	-
	40	++++	++++	-	-		-	-	-	-
	80	++	++	-	-		-	-	-	-

Thus, it was not possible to observe any relationship between neoplastic development and the rats' ability to participate in the complement fixation reaction with rat antigens.

When an attempt was made to trace how the properties of the sera of individual rats changed during the neoplastic development, it was found that as the neoplasm developed in some rats, the serum acquired the ability to give a complement fixation reaction with rat antigens (Table 2), while in others, on the contrary, it lost this ability, and in the majority of the rats the serum both before infection with the neoplasm and during its development gave a negative reaction.

Thus, study of the complement fixation reaction of rat sera before infection with the neoplasm and after it by the long cold method did not allow establishment of the appearance of specific antibodies in the rat sera during the process of neoplastic development.

Proof of the specifity of the antibodies in the rat sera by the absorption method was also unsuccessful (preliminary data).

For the absorption experiments, sera which gave a positive complement fixation reaction with rat antigens were taken. Absorption was carried out by splenic and hepatic tissues preserved in 5% formalin and alcohol [5].

From the data presented in Table 3 it is apparent that, during the absorption of the sera, a regular decrease occurs in the antibody titer with respect to all antigens with which the serum reacted before absorption, or they completely disappear.

The data which were obtained indicate that the complement fixation which we discovered is, apparently, nonspecific.

Thus, our investigation did not give convincing data to indicate that specific antineoplastic antibodies appear in the sera of rats during development of sarcoma M-1 in them.

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SUMMARY

The immunological properties of the serum of normal rats and of rats infected with sarcoma M-1 were pared at different lengths of time after infection. The prolonged cold complement fixation test was used.

No convincing evidence proving the appearance of specific tumor antibodies in the serum of rats inoculated sarcoma M-1 was observed.

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EXPERIMENTAL BIOLOGY

ON THE ROLE OF DESOXYRIBONUCLEIC ACID IN THE VERTEBRATE FERTILIZATION PROCESS

K. K. Sergeev

From the Chair of Histology of the Chita Medical Institute

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The investigation was carried out on representatives of various classes of vertebrates. The gonads of 7 bony fish (golyana), of 19 anurous amphibians (green toads), of 8 caudate amphibians (4 crested salamanders and 4 axolotls), 10 birds (5 sparrows, 2 strixes, 3 wild teal ducks), 9 mammals (6 white mice, 3 cats) and 3 humans. The preparations were stained by means of the Feulgen reaction to demonstrate desoxyribonucleic acid (DNA).

In investigating the testes, we observed that the nuclei of the spermatozoa were completely saturated with DNA in all cases. At the same time the nuclei of the spermatogonia and spermatocytes stained more faintly and contained DNA in smaller concentrations than the spermatozoa. During the process of spermatogenesis, the concentration of DNA increased especially sharply in the spermatids and spermatozoa in the progression of "spermatogonia-spermatocytes-spermatids-spermatozoa".

Fig. 1. Tissue from the gonad of the tadpole of the green toad. Desoxyribonucleic acid is absent in the nuclei of the oocytes.

During investigation of the ovaries of sexually mature fish, amphibians and birds, the absence of DNA was noted in the nuclei of the oocytes. Along with this, we observed that in three preparations in which the oocyte nuclei did not stain at all by Feulgen's method, the nuclei of the follicular cells surrounding the oocytes stained intensively and specifically, indicating that they contained a considerable amount of DNA.

At early stages of embryogenesis in the caudate amphibians (axolotl) and anurous amphibia (green toad), the oocyte nuclei contained DNA, but in considerably smaller concentrations than the nuclei of the follicular cells surrounding them. At later stages of development of the caudate amphibians, the DNA content of the oocyte nuclei decreased and by the end of metamorphosis the oocyte nuclei did not stain at all when Feulgen's reaction was carried out (DNA was lacking in them). The nuclei of the follicular cells surrounding the oocytes stained intensely and, consequently, contained DNA in considerable concentrations.

When studying the ovaries of mammals it was found that oocytes at the graafian follicle stage do not contain DNA, while the nuclei of the follicular cells surrounding them contain a considerable amount of this substance.

In the nuclei of oocytes an insignificant amount of DNA is contained at the primary follicle stage, at a much lower concentration than in the nuclei of the follicular cells surrounding them. A large amount of DNA is contained in the nuclei of the connective tissue cells of the gonadal stroma (Fig. 1, 2).

Fig. 2. Testicular tissue of the green toad. Nuclei of the spermatozoa are saturated with desoxyribonucleic acid.

Fig. 3. Medulla oblongata of a green toad tadpole. The chromosomes of the cells undergoing division are saturated with desoxyribonucleic acid.

From the above data, the following can be concluded:

1) Mature male and female mammalian germ cells differ sharply from each other in the DNA content of the nuclei; the nuclei of spermatozoa are completely saturated with DNA, while DNA cannot be found in the nuclei of mature oocytes.

2) During the process of spermatogenesis and oogenesis, contradictory phenomena are observed; the DNA content in the male germ cells increases, reaching a maximum in the spermatozoa; in the female germ cells, the DNA concentration decreases, reaching such an insignificant amount that it cannot be found with Feulgen's reaction.

3) During the fertilization process of mammals, the spermatozoon brings DNA into the egg cell, which, probably, has great significance, especially for the beginning of cell division.

We can express a definite hypothesis regarding the role of DNA in fertilization on the basis of our own data and that found in the literature.

In studying the metamorphosis of anurous amphubians, the author [8] observed that during the period of karyokinesis in the chromosomes, DNA is accumulated.

When the nuclei of the various tissue cells are compared [8] in preparations stained by Feulgen's method, it becomes clear that they contain different concentrations of DNA. It is interesting to note that the more DNA there is in the nuclei, the smaller the nucleoli are and vice versa. The tissue cells can be divided into 5 groups according to the size of the nucleoli and the concentration of DNA, of which the second and third groups are little differentiated from each other.

First group. Nuclei maximally saturated with DNA, the nucleoli cannot be located. Spermatozoa are included in this group.

Second group. Nuclei contain DNA in large concentrations. Nucleoli small, 0.8-1μ in diameter. Histocytes, the lymphoid cells of the thymus, the endothelium of the capillaries, the epithelium of the renal corpuscles, the epithelium of the thyroid gland, of the olfactory organ, the smooth muscle cells, the neuroglia are included in this group.

Third group. The nuclei contain DNA in smaller concentration than the nuclei of the preceding group; the diameter of the nucleoli is 1.5-2μ. The striated muscle fibers, the intestinal epithelium, the epithelium of the renal tubules, the cutaneous epithelium, the hepatic epithelium, epithelium of the salivary glands and of the efferent ducts of the exocrine portion of the pancreas are included in this group.

Fourth group. Nuceli are poor in DNA. the nucleoli are large, 2.5-4μ in diameter. The large neurons of the spinal cord and medulla oblongata, the neurons of the spinocerebral ganglions, the secretory cells of the exocrine sections of the pancreas belong in this group.

Fifth group. The nuclei do not contain DNA, the nucleoli are very large, 10-11μ in diameter. The ovarian oocytes compose this group.

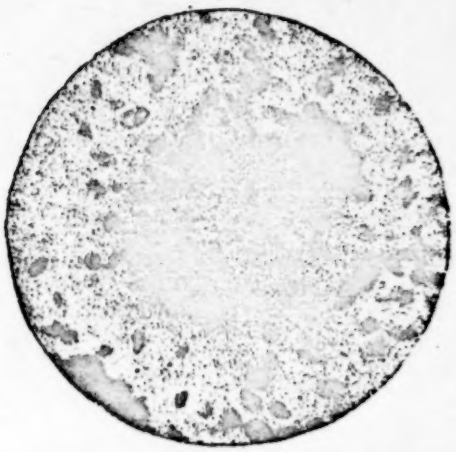

Fig. 4. Tissue from the tail of a green toad tadpole. Nuclear bodies, arising from the disintegrating nuclei, are saturated with DNA.

If the tissue cells of the above groups are analyzed from the point of view of frequency of mitotic division found among them, it is found that mitosis is encountered more frequently in the groups with large concentrations of DNA in the nuclei. On the contrary, the poorer the tissue cells are in DNA, the more rarely mitosis is encountered among them. The spermatozoa are an exception in this case, which can be explained by the fact that the DNA in their nuclei is in an inactive bound state until fertilization [18].

In investigating the organs of amphibians which undergo considerable destructive changes (tail, intestinal tract) during the period of metamorphosis, it was discovered that the DNA concentration increased in the nuclei not only in connection with cell division, but also in connection with the destruction of the cells during pycnotic changes in the nuclei. On the basis of the above, the hypothesis was made by us that DNA is a stimulator of biochemical reactions which occur both during cell division as well as during their destruction. In connection with this hypothesis, another was stated according to which the spermatozoa introduce DNA into the egg cell during fertilization, stimulating the process of cell division [8, 9].

In studying the literature on this problem, we found that many authors, even in old investigations, indicated the important role of the spermatozoa in the beginning of cell division; some considered that the spermatozoon brings the cellular center into the egg cell, others said that the sperm acted catalytically during contact. In the work of the last few years, indications can be found that the changes which occur during fertilization and cell division are dependent on the secretion of some kind of active substances by the chromosomes or head of the spermatozoon.

The authors of other papers speak of a change in the DNA content after fertilization, but do not connect this with the DNA in the spermatozoon. Swift and Kleinfeld [23] indicate the doubled amount of DNA in the nuclei of the segmenting eggs of the locust. Ludwig [19] described the change in DNA during the segmentation process in rats and khomyaks. During the prophase of segmentation, the author noted an accumulation of DNA, while the DNA disintegrated after the completion of cell division. At the 8-cell stage, the greatest amount of DNA was found in the nuclei. As the DNA content of the nuclei increased, a decrease in the size of the nucleoli was noted, which corresponds with our data also [8].

P. V. Makarov [4, 5], who studied the fertilization process of equine ascarids, described first a decrease, then an increase in the DNA content of the nuclear proteins. Braden and Austin [14] noted an increase in the DNA concentration during segmentation in rats. Brashe [13], investigating the development of amphibia, showed that 90 hours after fertilization the amount of DNA increased 26 times in the egg. All the above investigations confirm our views regarding the significance of DNA in the segmentation process.

The work of Horstadius [17] provokes special interest. When DNA was injected inside the unfertilized ovum of the sea urchin and it was mixed with the cytoplasm of the ovum, the ovum was activated and formed a fertilization membrane. Horstadius also notes that at low DNA concentrations the fertilization membrane is not formed.

In a number of papers there are indications that DNA is the active stimulator of the cell division processes both during segmentation as well as under other conditions.

Lison and Pasteels (cited from Zh. Brashe [12]) communicate that the DNA content increases in areas of intensive cell division.

K. Lang [18] writes that the formation of DNA occurs only during cell division. A. Bendich [11] and Zh. Shmerling [10] note that the intensity of the DNA metabolism increases during regeneration.

P. V. Makarov [5] noticed a regular increase of DNA in the nucleus during prophase and a decrease of it during telophase.

With respect to the DNA content of the germ cell nuclei, the literature data vary somewhat.

Pollister [21] indicates that the haploid nucleus of the spermatozoon and egg cell contains half as much DNA as the nuclei of somatic cells. The DNA content of one type of somatic cell in the organism is the same.

Dalcq [15] notes that DNA is found in a considerable concentration in the nuclei of the somatic cells of the mammalian ovary, including also the nuclei of the follicular cells. In the oocyte nuclei, according to A. Dalcq, the DNA is localized in the finest chromosomes.

Our own data, presented above, indicate that DNA is distributed differently in the nuclei of the various tissue cells of one species of animals (anurous amphibia) which does not correspond with the data of a number of other authors. At the same time, our data regarding the various distribution of DNA in the tissue cells found confirmation in some papers.

Pasteels and Bullough [20] noted that there is less DNA in the superficial cells of the epidermis of mice than in the lymphocytes, and that the DNA concentration in the growing layer of the epidermis is higher on the average than in the lymphocytes.

Zh. Brashe [12] cites the investigations of Lison and Pasteels (1951) of the change in the DNA content during the ontogenesis of the sea urchin. According to the data of these investigators, marked differences appear between the nuclei of various tissue cells with respect to DNA content.

Thomson and Frazer [24] note that 8 times as much DNA can be present in the rapidly regenerating liver nuclei than in normal ones.

Our data indicate that during the course of spermatogenesis and oogenesis, the DNA content of the nuclei changes in different ways, and not in the same way, as confirmed in some of the papers cited above.

According to our data, DNA, which is necessary for fertilization and egg segmentation, is introduced by the spermatozoon, whose nucleus contains it in maximum concentration. In some work it is possible to find confirmation of our data. Thus, D. Devidson [16] notes that DNA can compose 48.5% of the dry weight of the fat-free fraction of the sperm heads. P. V. Makarov [6] indicated that the sperm heads consist of nucleoprotein, which is in a condensed state, and are not packages of chromosomes. B. V. Kedrovsky [3] supplies data on the high DNA content of the nuclei of young spermatocytes of the grasshopper.

The feasibility of parthenogenetic development, in which segmentation occurs but DNA is not introduced into the egg cell from outside, contradicts at first glance our point of view regarding the role of DNA in the fertilization process of vertebrates. However, the role of DNA cannot be denied on this basis, as the role of the spermatozoon in the fertilization process cannot be denied just because it does not participate in parthenogentic reproduction.

It is possible to suggest that in the parthenogentic process, a change of RNA to DNA occurs in the egg cell, which stimulates segmentation. At the present time there are works in which the possiblity of changing RNA into DNA under certain conditions has been demonstrated. Thus, as early as 1931, Brashe [12] showed that during the development of sea urchin eggs an increase in the DNA content occurs while the cytoplasmic RNA decreases. G. I. Roskin [7] described the change of RNA into DNA during mitotic division.

Zh. G. Shmerling [10] states that disappearance of mitoses leads to a decrease in the amount of DNA in the tissues and to an increase in the RNA content.

B. I. Goldshtein and co-workers [1, 2] established the change of RNA into DNA in the tissues of the sea urchin outside of the organism under the influence of Vitamin C.

On the basis of all the above, the following can be stated: DNA is a chemical compound which stimulates biochemical reactions which occur during cell division. During fertilization, the spermatozoon introduces DNA into the egg cell, which creates in the zygote the DNA concentration necessary to stimulate the segmentation processes. In the case of parthenogentic reproduction, an increase in the DNA concentration at the expense of the RNA in the egg cell under the influence of external agents may be hypothesized.

SUMMARY

It was established that the amount of DNA in the nuclei of the cells of male gonads of mature fish, amphibia (Anura and Urodela), birds, mammals and man greatly increased in the process of spermatogenesis. Especially great amounts of DNA were found in the nuclei of spermatozoa. In mature oocytes, in contrast with the follicular cells, no DNA was found. The author believes that DNA, which plays an important part in the development of the fertilized egg is brought into the oocytes by spermatozoa.

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ON THE REGENERATION OF THE NEURONS OF THE INTRAMURAL PLEXI OF MAMMALS

N. M. Shestopalova

From the Laboratory of Growth and Development (Head – Prof. M. A. Vorontsova) of the Institute of Experimental Biology (Director – Prof. I. N. Maisky) of the Academy of Medical Sciences of the USSR, Moscow

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The problem of the development and regeneration of the neurons of adult mammals is extremely complicated and very difficult. It was considered established that neurons, once developed, function throughout the life of the organism, without being replaced by new ones when old or injured.

Most authors explain the inability to replenish neurons by a complicated morphological and functional differentiation and by the absence of a cambial source in nervous tissue [14, 3].

Some authors concede the possibility of the existence of cambial cells in nervous tissue [8, 11]. Due to development of these cells during the postnatal period, dying neurons of the adult organism are replaced. The amount of cambial cells depends on the age, the area of the nervous system, and on the systemic condition of the animal.

The widely known ability of epithelial and connective tissue to regenerate was explained by their more ancient origin and by their uncomplicated morphological and functional differentiation. But the tissues which appeared at later stages of phylogenetic development were considered incapable of regeneration. Included among these were muscular (smooth and somatic) and nervous tissues.

However data are available indicating the inapplicability of this concept. It is known that the smooth muscles of the blood vessels [4], uterus [19], and intestines [15, 4] are able to regenerate. Their regeneration occurs by amitotic and karyokinetic division of the smooth muscle cells and myoblasts. Equally able to regenerate [12] is skeletal muscle. The removal of a muscle under certain conditions is accompanied by its regeneration.

The ability of the spinal cord to regenerate, i.e., the nervous tissue, during regeneration of the tail in lower vertebrates, has been described [17, 2, 6]. Differentiation of neuroblasts and development of ganglions take place in the embryonic brain tissue of birds when cultured outside of the organism [5]. The ability of the peripheral nerve process and endplates ro regenerate, as well as the neogenesis and growth of new processes of neurons, is widely known in the higher vertebrates [1, 18, etc].

In the present work an attempt was made to discover what happens to the neurons of the intramural plexi of the intestinal tract when the organism is injured [1, 18, etc.].

EXPERIMENTAL METHOD

The work was carried out on 120 white rats. The operation was carried out by the method worked out earlier [15]. The operated area was carefully marked by surgical silk in the form of knots on the four sides of the wound. The material was fixed immediately after the operation, 3, 24, 48, 60 hours later, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 20, 23, 27, 28, 30, 42, 54, 63, 68 and 70 days after infliction of the wound. As fixatives were used 12% neutral formalin and Zenker's fixing fluid with added formalin. The impregnation was

carried out according to Buke and Bielschowsky-Gross with subsequent gold staining. The series of paraffin sections was stained with toluidine blue in order to show the tigroid substance, hematoxylin with additional staining with picrofuchsin, eosin blue, and according to Feulgen.

Two series of investigations were carried out. In the first series, a penetrating wound with an incision 4x3 mm^2 was inflicted on the animal, in the second an area of the intestinal wall 4x7 mm^2 in area was crushed to the state of a thin transparent film (by compressing it many times with Pean's forceps).

EXPERIMENTAL RESULTS

Study of the operated area and the zone adjacent to it showed that liquidation of the wound defect occurred in 10-15 days. At the same time, completere-establishment of the removed portion of the intestinal wall took place. The external surface of the operated area became smooth at the end of the second week and was covered with serosa. Villi were seen on the inner surface of the mucous membrane when examined with a stereoscopic magnifying glass in reflected light. They were located throughout the entire operated area and differed only in the center from the surrounding ones by their lesser height. The defect in the muscular coat was filled in by regenerating smooth muscle tissue. Regenerating intramural plexi were noted in the newly-formed smooth muscle tissue in the injured area.

The regeneration of all portions of the intestinal wall took place by means of regeneration of the tissues from the edges of the wound.

When a penetrating wound was inflicted, the edges of the wound remained separate during the first days. The space between the edges was filled with effused blood. In addition to fibrin and blood elements, there are destroyed tissues in the wound. Later, the fibrin undergoes organization, the remains of the destroyed tissues become necrotic and undergo resorbtion in the process of the developing inflammation. The inflammatory process spreads to tissues adjacent to the wound also. Degenerative phenomena are observed in the tissue cells of the border zone. The connective tissue surrounding the nodes of the intramural plexi becomes separate fibers. In part of the ganglion cells of the nodes is observed wrinkling of the cell body, vacuolization of the protoplasm, argentophilia increases, staining with toluidine blue by Nissl's method increases. The nuclear membrane becomes more sharply and coarsely evident. The fine structure of the nucleus is destroyed. Dark lumpy nuclei appear when stained according to Feulgen. Degenerative phenomena in the ganglions of the zone surrounding the wounded area are observed during the first week, individual cases are found at later dates as well. Simultaneously with the degenerative processes in the uninjured tissues and in those not involved in the inflammatory process, reactive changes arise: the cellular elements increase in size, the ability to stain with basic dyes appears or this ability increases, the ability to proliferate appears, the fine structure of the cellular elements changes. Regenerative processes begin at the tissues which changed reactively. Connective tissue and blood-vessels grow. An intensive process of amitotic division of the smooth muscle cells is observed in the muscular coat during the first two days. On the second day, a large amount of mitotic division of the smooth muscular cells and myoblasts is noted. The myoblasts which are formed move from the edges to the center of the wound, thus liquidating the defect in the muscular coat.

Simultaneously with the movement of the regenerating smooth muscle tissue, the regenerative processes develop in all the structural components of the intestinal wall. At the ends of the cut fibers of the nerve plexi 60 hours after the operation growth bulbs are evident. Later growth bulbs appear in the tissues filling the wound. An excess amount of chromatin is noted in the nuclei of the nerve cells of the ganglions in the bordering zone. Frequently nuclei with 2-3 nucleoli occur; oval nuclei and those contracted in the middle occur. The structure of the cell body changes; a light area of neuroplasma appears near the nucleus, the appearance of protoplasmic nodules is observed in the peripheral areas of the cell; a large number of binucleated neurons and cells connected by bridges are present.

On the 7th day of regeneration after injury, in the nodes of the intramural plexi were observed mitotic division of the ganglion cells, various stages of karyokinesis were observed (Fig. 1-3). Later mitosis was observed on the 10th and 11th day also. Neurons appeared between the regenerating muscular layers and in the tissues filling the wound. They were located at first singly, then their number increased and rods of 3-4 cells appeared. Amitotic division was observed in the cells of the rods.

Fig. 1. Ganglion cell of a perivascular plexus. The peripheral location of chromatin in the nucleus can be seen; beginning of mitotic division. Zenker's formol, eosin blue. Microphotograph. Enlargement: ocular 5x, objective 100x.

Fig. 2. Ganglion cell from the node of Auerbach's plexus in a state of mitotic division. Prophase, beginning of chromosome orientation in the equatorial plane. Zenker's formol, eosin blue. Microphotoraph. Magnification: ocular 5x, objective 100x.

On the 8th day proliferating nerve fibers and neurons, similar in structure and location to the cells of Meissner's plexus, were observed in the tissues of the wounded area. By the 10th day, rods of 5-6 and more cells were observed in the tissues filling the wound. In transverse sections, their location along the developing muscular layers was seen. Several groups of nerve cells were evident near the regenerating blood vessels of the mucous membrane in stained longitudinal sections through the wounded area. Some of them lay alone, others were interconnected into nerve fibers. On the 20th day, accumulations of nerve cells were found between the newly-formed muscular layers. The nerve cells were joined into ganglions, which were connected by nerve fibers.

Thus, cell division was found in all the regenerating tissues. The time when the dividing cells appeared in the various tissues and their number varied. We observed karyokinetic division of the smooth muscle cells and myoblasts in large numbers beginning on the second day; division of the neurons was noted beginning with the 7th day after injury.

Attention should be paid to the fact that the regeneration of the ganglionic apparatus proceeds simultaneously and parallelly with the regeneration of all the components of the intestinal wall. The external muscular and serous coats regenerate. The regenerated smooth muscle tissue does not have the correct separation into the longitudinal and transverse layers at early stages of development. First a single layer of muscular tissues is formed in which the smooth muscle cells are not oriented. During the regeneration of the smooth muscular tissue, myoblasts appear which have the ability to divide mitotically.

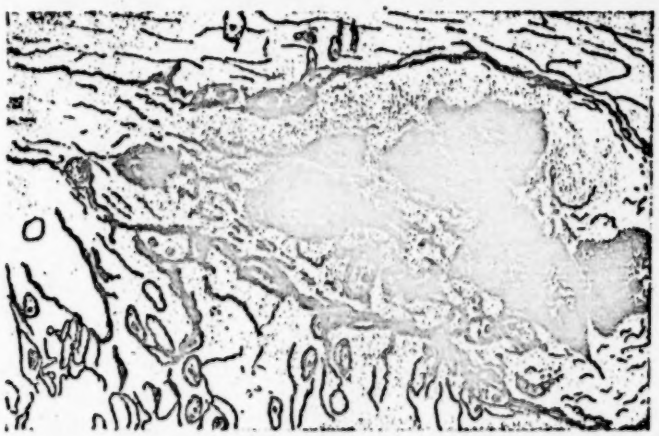

Fig. 3. Ganglion cells of Auerbach's plexus in a state of mitotic division. Metaphase. Beginning of chromosomal movement to the poles. Zenker's formol, toluidine blue. Magnification: ocular 5x, objective 100x (drawn with Abbe's drawing apparatus).

In the process of regeneration of the intestinal wall, regeneration of the mucosa takes place, including regeneration of the normal villi. The regeneration processes of the structural components of the intestinal wall are interrelated and ensured by their simultaneous development as units of a single whole.

Summarizing the results which were obtained, it must be observed that the regeneration processes which take place when a penetrating wound is inflicted and on crushing proceed in a similar way in general. The difference consists of the fact that regeneration of the intestinal wall in some cases takes place a few days earlier. The reason for this, apparently, is that in these cases undestroyed collagen fibers remain sometimes at the place where the defect was made. Apparently, they serve as a channel for the regenerating cells and so speed the entire regenerative process.

Analyzing the results which were obtained and correlating them with the data available in the literature, it should be noted that information has appeared in print during the last few years which indicates the possibility of the multiplication of neurons in adult organisms of higher vertebrates.

V. V. Troitsky, M. V. Rudenskaya [13], L. B. Levinson and M. M. Leikina [9], Z. S. Katsnelson [7], A. F. Nikiforov [10] and others found amitotic division of the neurons of the extramural ganglions and of the brain.

Thus, regeneration of the nodes of Auerbach's and Meissner's plexi takes place after infliction of trauma on the wall of the small intestine of white rats. In the ganglions in the zone surrounding the wounded area reactive changes occur which lead to an increase in the number of neurons. In the edge and regenerated zones, karyokinetic and amitotic division of the neurons is noted. In addition, in the regenerated muscular tissue of the external muscular coat and near the regenerated blood vessels, nodes of the intramural plexi are found.

Consequently, the intramural ganglionic apparatus of the intestinal wall of higher vertebrates has the ability to regenerate.

SUMMARY

After the infliction of deep and contused wounds on the wall of the small intestine of white rats regeneration of the ganglia of Auerbach's and Meissner's plexi took place along with the restoration of the principal structures of the intestinal wall. Karyokinetic and amitotic division of neurons were noted in the zone surrounding the wound as well as in the regenerated zones.

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REGENERATION OF THE PAROTID GLAND OF A GUINEA PIG

A. G. Babaeva

From the Laboratory of Growth and Development (Head – Prof. M. A. Vorontsova) of the Institute of Experimental Biology (Director – Prof. I. N. Maisky) of the Academy of Medical Sciences of the USSR, Moscow

(Received September 14, 1956. Presented by Active Member of the Academy of Sciences of the USSR N. N. Zhukov-Verezhnikov)

The regeneration of salivary glands has served as the subject of investigation a number of times.

Podwyssozki [9] (1887), Ribbert [10] (1895), Carraro [7](1909), Milstein [8] (1950), Gerlovin [3] (1955) in experiments on the submaxillary salivary gland of rabbits, rats and cats proved its ability to regenerate. It is considered to be established that the regeneration of the salivary gland takes place due to the proliferation of its ducts, and that the newly formed tissue is not functionally of full value.

The ability of the parotid gland to regenerate has hardly been studied. In a single investigation, belonging to Koropov [4](1949), the conclusion is made that the parotid gland of dogs is unable to regenerate.

The parotid gland of the guinea pig was selected by us as the subject of investigation.

The regenerative ability of the parotid gland was studied when the paired organ was removed, which, according to literature data, facilitates regeneration–Ingle and Higgins [6] (1939), Artemyeva [1] (1951) The right salivary gland was always removed. The left parotid gland was resected at the level of the lower edge of the lower jaw. In order to study the histological structure of the regenerating gland, the animals were killed 1, 3 and 7 days after the operation and at various lengths of time after the operations up to one year. The glands were fixed by the usual histological fixatives and embedded in paraffin. In all, 17 animals were used.

As study of the histological preparations showed, deep destructive processes are observed after partial resection of the gland in the area directly adjacent to the place where the cut was made. Along with this, weaker degenerative changes in the secretory cells even in areas distant from the cut were found. The maximum changes were observed on the 3rd day after operation. By the 7th day, the indicated degenerative changes disappear.

From the first days after the operation, regenerative processes occur in the gland together with destructive ones. They are first evident in the appearance of a greater number of mitoses in the secretory cells of the terminal areas and in the cells of the principal excretory duct. As a result of the intensive proliferative processes which occur, an enlargement of the remaining portion of the gland is observed. Even by the end of the first month after the operation the regenerating gland approaches the glands of the control animals in its absolute and relative weight. The same phenomenon is characteristic of later dates of fixation also. The relative weight of the salivary glands of the experimental animals was 0.107%, i. e., it reached the average value of the relative weight of the salivary glands of the control animals of the same weight (0.099%) or of the same age (0.107%).

In addition to the increase in size, an enlargement of the linear dimensions of the gland was also observed. Before the operation, the distal end of the gland was 1 cm below the edge of the jaw on the average. By the end of the first month after the operation, the distal edge of the gland was on the average 0.5 cm below the edge of the jaw, i. e., below the amputation level.

In histological investigation and graphic reconstruction of the gland it was found that the area of the gland which is located distal to the level of the amputation consisted of 2 parts: one part was of completely normal structure, the second, located around the principal excretory duct, had atypical structure. The latter is well described by many authors who turned their attention principally to this part of the gland when studying regeneration.

The atypical structure of the part of the gland which is adjacent to the duct is evident in the fact that its terminal sections consist of 8-16 cells, while in the normal gland they consist of 3-8 cells usually. Their size is greater: thus, 10-15 terminal sections fit into one field of vision (ocular 7, objective 40) of the normal gland, while in the atypical part of the gland, only 4-6 fit. The sizes of the cells in the terminal sections of this part of the gland are considerably less than in the terminal sections of the normal gland.

The presence of normal glandular tissue below the level of the amputation cannot be explained, as we believe, by the fact that the initially atypical structure of the regenerating gland assumed normal structure. This hypothesis is unlikely, since we did not observe transitional forms between the terminal sections with atypical and normal structure and, in addition, part of the gland which is adjacent to the principal excretory duct preserves its atypical structure for a long time. The assumption remains that the regeneration of the gland is only partial and takes place in only an insignificant degree due to the proliferation of the ducts, but chiefly to the regenerative hypertrophy—Vorontsova [2], (1953) i. e. due to multiplication of the secretory cells and to the formation of new acini. The fact that a comparatively large number of mitoses is present in the secretory cells, which does not lead to a noticeable increase in the number of cells in the terminal section nor to an increase in their dimensions, indicates this. Since the tissues surrounding the gland adjoin it closely, the increase in linear dimensions occurs chiefly downward, where the excised area of the gland was previously located.

The majority of authors consider that the regenerated portion of the gland is unable to function. This statement requires detailed verification. In the light of our data, this problem should be subdivided. As regards that section of the gland which preserves a normal structure, its function, apparently, does not arouse doubt. As regards the section which is of atypical structure, according to our observation, the ducts of this portion of the gland always contain some amount of secretion. The latter circumstance indicates that the regenerated cells secrete, although it is difficult to say on the basis of morphological data how complete their functional activity is. The secretion also has the ability to overflow into the oral cavity, since the ducts of the regenerated area are connected with the duct of the normal part of the regenerated gland, as data from the graphic reconstruction of the glandular ducts indicate.

The data we obtained show that the parotid gland of guinea pigs has a fairly high capacity to regenerate. This is evidenced in the fact that the original weight and dimensions of the gland are re-established within a comparatively short time (within a month). The regeneration occurs basically due to the formation of glandular tissue which has the same structure as the normal.

At the same time, the observed regeneration cannot be regarded as fully complete. Part of the regenerated gland which is adjacent to the excretory duct preserves an atypical structure. In addition, complete regeneration of the original shape of the gland is not observed.

The fact that the regenerated gland consists of two heterogeneous parts allows explanation to a certain extent of the statements of the majority of authors who consider that the regeneration of the gland occurs due to proliferation of the ducts and that the regenerating gland is not functionally fully complete. Apparently, these authors turned their attention primarily to that part of the gland which is characterized by atypical structure.

SUMMARY

After the removal of the right parotid gland in guinea pigs and partial resection of the left gland, the regeneration of the left gland was observed. Three weeks after operation the resected gland recovered its initial weight and size, chiefly through the proliferation of secretory cells in the terminal areas and the formation of glandular tissue of normal structure. The part of the gland adjacent to the main secretory duct, regenerated by means of proliferation of the glandular ducts, and for a long time retained atypical structure.

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MORPHOLOGY AND PATHOMORPHOLOGY

THE PROPRIOCEPTORS OF THE OCULAR MUSCLES OF MAMMALS

M. A. Kalugina

From the Chair of Histology (Head – Honored Scientist Prof. A. N. Mislavsky) of the Kazan Medical Institute and the Chair of Histology (Head – Prof. M. Ya. Subbotin) of the Novosibirsk Medical Institute

(Received July 12, 1956. Presented by Active Member of the Academy of Medical Sciences of the USSR V. N. Ternovsky)

The problem of the existence of neuromuscular fibers in the rectus oculi muscles has remained unclear until the present. Data are lacking in the literature to characterize the interrelation between the process of the development of proprioceptors and the functioning of the organ, in particular with the development of vision.

The most detailed description of the sensory apparatuses of the ocular muscles was made by A. S. Dogel [1]. A. Cilimbaris [4] and A. S. Dogel described typical neuromuscular fibers in the ocular muscles. Other authors sought the fibers in the ocular muscles unsuccessfully [5]. S. Mikhailov [2] stated that the sensory neural apparatuses of the heart and ocular muscles should be of one type, since they accomplish the very "complex and active play of contractions." In recent investigations [3] also no indications exist of the neuromuscular fibers in the ocular muscles.

Fig. 1. Sensory nerve ending in the ocular muscles of a cat. Treated according to Gross-Bielschowsky. Ocular 10x, objective 90.

The present investigation represents an attempt to discover whether the neuromuscular fibers exist in the ocular muscles of animals and to establish a connection between the stage of development of the sensory apparatuses and the functioning of the eye (visual development).

EXPERIMENTAL METHOD

We studied the rectus oculi muscles of some mammals of various age groups: cats (newborn, four-day old, two-week old, adult); dogs (four-day old, two-week old, two-month old); guinea pigs (newborn, two-week old, two-month old). The animals chosen were born blind (cat and dog), as well as with sight (guinea pig). The material was treated by the Gross-Bielschowsky method.

EXPERIMENTAL RESULTS

In many investigations of the ocular muscles of adult animals, the neuromuscular fibers were not found. Sometimes a spiral arrangement of the nerve fibers could be observed in cats, but this was not an ending and

there was no connective tissue capsule around these formations. All the sensory nerve endings which were found were of the free type.

The sensory nerve endings in the ocular muscles of various animals differed considerably.

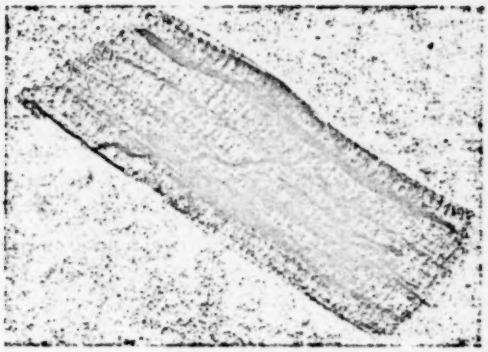

Fig. 2. Sensory nerve endings of the ocular muscles of an adult dog. Gross-Bielschowsky stain. Ocular 10x, objective 90.

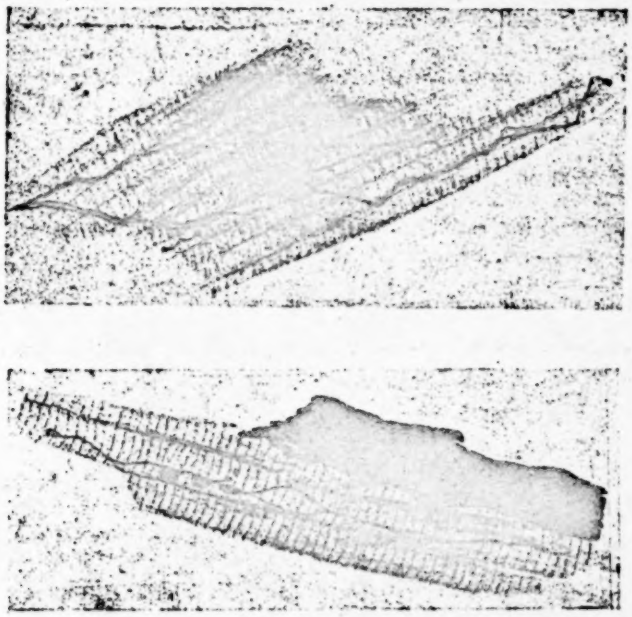

Fig. 3. Sensory nerve endings of the ocular muscles of the guinea pig.
Newborn (above); two-week old (below). Treated according to Gross-Bielschowsky. Ocular 10x, objective 40.

In the adult cat, the endings on the muscle fibers were in the form of branching fibrils, extending along the muscle fibers, with many varicose thickenings and areas of ramification (Fig. 1). In addition to such endings, less complex ones were found which represented a fine thread with varicose thickenings.

The proprioceptors in the rectus oculi muscles of an adult dog had the appearance of branching bushes with many varicose thickenings. The branches of the bushes were directed along the muscular fibers (Fig. 2).

In studying the rectus oculi muscles of adult animals (the cat, guinea pig, dog) some zoning was established in the location of nerve endings. The motor endings were located in a more or less limited zone. The sensory endings could be found in various areas of the muscle. They were found in the zone of the motor endfeet as well as in areas poor in motor endings. As observations showed, in the process of the development of the proprioceptors of the ocular muscles, a gradual complication of the nerve endings occurs – with age they become less diffuse and occupy lesser areas of innervated tissue.

On comparing the stage of differentiation of the sensory nerve apparatuses of animals born blind and of animals born with sight, a certain difference can be found. Thus, the nerve endings of a newborn cat are very fine threads with many varicose thickenings which extend a considerable distance. The sensory nerve endings of the two-week old cat are just as simple. The complication of the endings occurs very slowly.

The differentiation of the endings occurs considerably more rapidly in animals which are born with sight (guinea pig). The sensory nerve ending of a newborn guinea pig are very delicate sprays with numerous terminal structures (Fig. 3a). The endings of two-week old guinea pigs are already more differentiated: more complex than in the newborn and more limited (Fig. 3b).

Thus, the differentiation of the nerve endings proceeds more rapidly among animals born with vision than among those born blind.

SUMMARY

The authors found no neuromuscular fibers in the ocular muscles of the investigated animals (cats, dogs, guinea pigs). The sensory nerve endings which were found were of the free type. They were found in the zone of the motor end-feet as well as in areas poor in motor endings. In animals born blind differentiation of the proprioceptors proceeded somewhat more slowly than in animals with sight.

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* In Russian.

DEVELOPMENT OF THE CONNECTIVE TISSUE STROMA IN THE FETAL PORTION OF THE HUMAN PLACENTA

V. P. Zhuk

From the Department of Histology and Embryology (Chairman – N. Ya. Subbotin) of the Novosibirsk Medical Institute

(Received August 5, 1956. Presented by Active Member Acad. Med. Sci. USSR V. N. Ternovsky)

Of all the placental tissues, the connective tissue framework of the chorion is the one least investigated. The attention of research workers has been drawn principally to the epithelium and, to a lesser degree, to the vessels of the fetal portion. Only within the last few years have there appeared studies presenting the characteristics of the connective tissues as seen in early human embryos [1, 3]. These investigators state that the earliest extraembryoblastic human mesenchyme (in the initial postimplantation presonite period at about the 13-15 day stage) is divisible into the amnionic and chorionic. Soon reticular fibers appear between the elements of the chorionic mesenchymal syncytium transforming this structure into reticular tissue. Within this latter, blood islets form – the commencement of intravascular hematopoiesis.

Information in regard to the connective tissue components, especially the cellular elements, of the human chorion in the later stages of development is quite scanty. Ulesko-Stroganova indicates alterations in the mesenchymal chorionic stroma as gestation progresses [4], the early undifferentiated connective tissue, quite embryonal in appearance, being (after the fourth month) changed by the development of a large amount of collagenous fibers. Indications of a considerable collagenization in the stroma of the chorionic villi in the later developmental stages are present in other papers also [2, 5]; however, none of these authors suggest that there occur changes in cellular elements of the villous stroma.

In so far as metabolic exchanges between the mother and the embryo require the participation of the chorionic connective tissue, it seemed desirable to undertake a systematic study of the fetal connective tissues composing the placenta and examine them at various stages of their development.

EXPERIMENTAL PROCEDURES AND THE RESULTS OBTAINED

As our material for study, we took mature human placentas and chorions at various stages of pregnancy (from 5 to 16 weeks). This material was used only after we had satisfied ourselves that there had been no pathological changes in the fetal membranes. The tissues were fixed in Zenker-formol and 12% neutral formalin; Heidenhain hematotoxylin or azure-eosin was employed to stain the cellular elements; the Mallory method was used to demonstrate the interstitial fiber substance.

At a stage corresponding to the 5-6th week of pregnancy, two types of chorionic villi were encountered each having a different structure of the connective tissue stroma: avascular (Fig. 1) and vascular (Fig. 2). In both, the connective tissue stroma has a reticular character; the avascular villi contain somewhat fewer cellular elements while the latter are less polymorphic.

The stroma of the avascular villi can be seen to be a many-branched syncytium of cells having very large nuclei occupying almost the entire cell body. The cytoplasm is clear, faintly basophilic; the nuclei are large, round or oval with evenly distributed clumps of chromatin. The nucleolus stands out distinctly within the nucleus. It is not possible to demonstrate free cellular elements. The matrix of these villi is represented by a thin

network of reticular fibers in the loops of which there lies a finely-grained substance staining bluish with Mallory. The avascular villi possess no collagen fibers that can be demonstrated.

The vascular villi are of two types. The villi stemming from the basic chorionic disc contain more reticular fibers, the villous center only having collagenous replacement. The cellular elements of these villi are represented by fibroblasts forming a syncytium and oriented parallel to the course of the villus. In comparison with the villi of the other type but few free cellular elements can be seen.

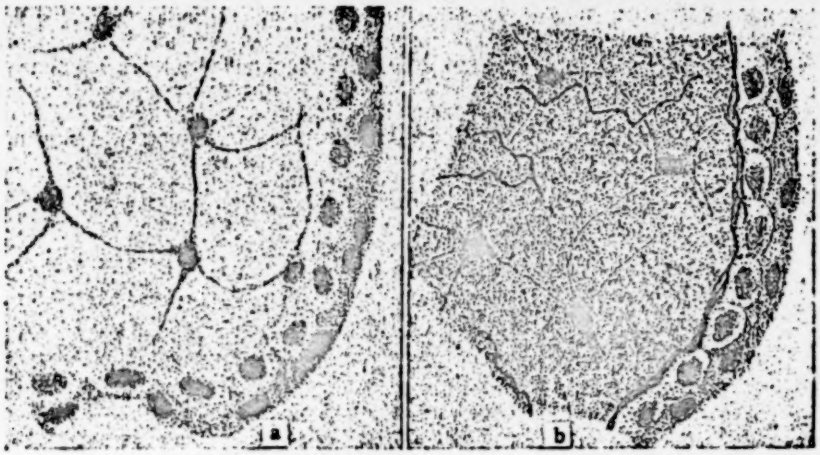

Fig. 1. Avascular chorionic villi (pregnancy 5-6 weeks). Enlargement: ocular 10x, objective 40.
Stain: a) Heidenhain iron hematotoxylin; b) Mallory method.

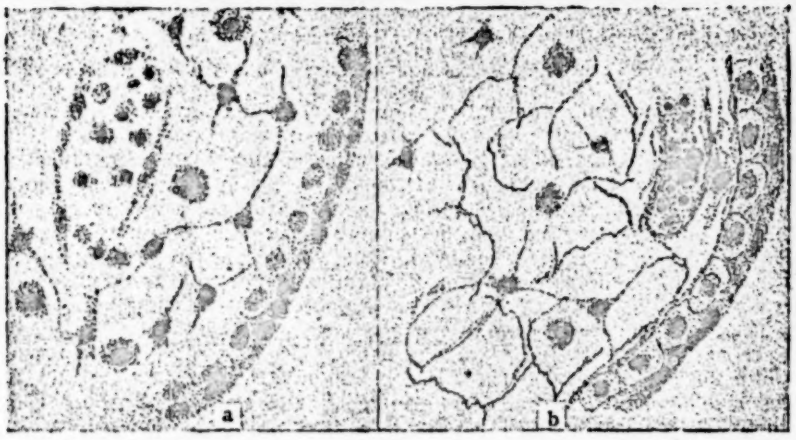

Fig. 2. Vascular chorionic villi (pregnancy 5-6 weeks). Enlargement: ocular 10x, objective 40.
Stain: a) Heidenhain iron hematotoxylin; b) Mallory method.

The stroma of the villi forming terminal branchings previously described has a syncytium of loosely-bound faintly basophilic fibroblasts very reminiscent of the cell elements seen in the avascular villi and has also large individual cells taking the basic stains with greater intensity. The nuclei of these free cells are round or oval, darker, and the clumps of chromatin are larger and less evenly distributed. The cellular cytoplasm appears

porous with uneven edges. These cells are from $1^1/_2$ to twice the size of the preceding cells. Morphologically, these free cells greatly resemble macrophages (histiocytes) seen in the loose connective fibrous tissue (see Fig. 2). The matrix of these terminal villi is represented by a fine net of reticular strands, completely unoriented. Only near vessels and at the boundary with the chorionic epithelium do these fibers form a reticular membrane composed of thicker strands. These villi are bare of collagenous fibers in the stroma. When the structures of the vascular and avascular villi are compared, it can be seen that the avascular type may be regarded as being the more primitive, composed of a syncytium of undifferentiated cells and a fine net of reticular fibers forming the primary villi which either go on and involute or else develop into the secondary type. The secondary villi are more complex, having many branches; at the base, in association with the chorionic plate, they constitute a differentiated connective tissue richly supplied with collagen fibers. The terminal branches are formed of less differentiated tissue rich in reticular strands and cells of the juvenile fibroblastic type, as well as free histiocytic elements.

At all later stages of development of the pregnancy, there are seen villi whose stroma at the base differs from its appearance in the terminal branchings; further descriptions of the connective tissues seen in the villi take this into consideration.

At still later stages of pregnancy, we failed to encounter the primitive avascular villi possessing a connective tissue stroma.

At approximately the stage corresponding to a 7-8-week pregnancy, the terminal branchings of the villi as described above manifest a sharp rise in the number of cellular elements, there being a large increase in free macrophagic cells, usually rounded, with round dark nuclei, porous cytoplasm and uneven edges. Clasmatocytes appear. Occasionally, two-nuclei cells are seen (Fig. 3). The second cell type, as before, is a syncytium of irregular small cells (fibroblasts). Their nuclei are oval or round, with nuclei of a lighter hue than that of the free cellular elements. In the fibroblasts mitoses are seen quite frequently.

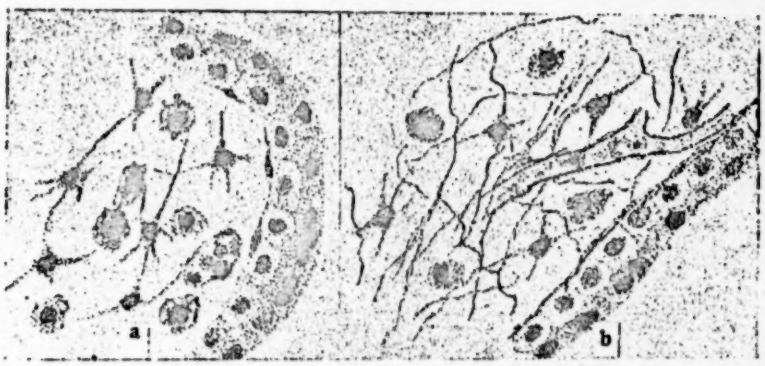

Fig. 3. Terminal chorionic villi (7-8-week pregnancy). Magnification: ocular 10x, objective 40.
Stain: a) Heidenhain iron hematoxylin; b) Mallory method.

At the very base of the villi, in close association with the chorionic plate, the cellular elements are fewer than further in the terminal branchings. Here one encounters principally fibroblasts and fibrocytes oriented along the long axis of the villus. In the central portion of the villus, they are especially numerous. Free, macrophage-type cells are not numerous in the basal divisions of the villi and in their disposition a certain order can be seen; they lie at the periphery of the villus, near the chorionic epithelium. Here in the matrix the collagen, not reticular, fibers predominate lying more frequently towards the center of the villus. In the central portion of the larger villi, as if ensheathed in a case of collagen fibers, lie the larger vessels-arteries and veins. The capillaries locate at the periphery being separated from the other tissues of the villus by a reticular membrane.

At the 9-10, and 12-12-week stages of gestation there is observed some diminution in the cellular elements and among the latter – a relative increase in the fibroblasts when compared with cells of the macrophage type.

The macrophage-type cells cannot be as definitely related to their earlier stages, the cytoplasm being somewhat more vacuolated and clasmatocytosis not being observed. The fibroblasts lose their continuity, amitotic

division becomes more common. In the matrix of the villi, collagenous fibers come to predominate, this being especially noticeable in the larger villi.

At the 15-16-week stage of gestation, the differentiation of the connective tissues is at its sharpest. At the bases of the villi the stroma consists principally of ground substance with a predominance of collagen fibers. Among the cellular elements elongated fibroblasts are most common; outgrowths are few, merging with the fibers of the intermediate substance. The nuclei of the fibroblasts are elongated on the long axis of the cell, becoming almost stick-like and staining rather intensively with the basic dyes. As the branchings of the villi are followed, the structure changes (Fig. 4): in the terminal twigs there is less of the ground substance, principally reticular fibers so that only around the vessels and in the central portions of the villi are collagen fibers to be observed.

Fig. 4. Terminal chorionic villi (15-16-week pregnancy). Magnification: ocular 10×, objective 40.
Stain: a) Heidenhain iron hematoxylin; b) Mallory method.

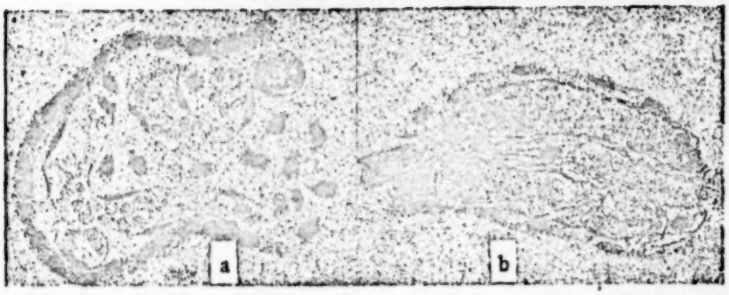

Fig. 5. Terminal chorionic villi (pregnancy 10 lunar months). Magnification: ocular 10×, objective 40.
Stains: a) Heidenhain iron hematoxylin; b) Mallory method.

The cellular elements in the terminal portions of the villi are more varied than they are at the base, the fibroblasts still predominating but differing considerably from the fibroblasts seen at the bases. These are multi-branching cells with oval or round nuclei, manifesting frequent amitoses. The cytoplasm of these fibroblasts stains so poorly that the cell boundaries are hard to define and syncytial-type union among the cells is not to be demonstrated. Free, histiocyte-type cells are here quite few, stain with greatly diminished intensity as compared to the earlier stages; the cytoplasm is markedly vacuolized. At the bases of the villi, as in the earlier stages, lie the larger blood vessels while in the branchings are the capillaries. They lie ensheathed in a fairly heavy layer of collagen (thicker at the villous base).

The structure of the connective tissues in the villi of a mature placenta at birth in principle is no different from the same tissue at the 15-16-week stage (Fig. 5). It is only important to note that, at term, the number of blood vessels increases sharply, the capillaries, in particular, dilating and assuming almost a sinusoidal appearance.

On the basis of the considerations presented above, we can conclude that the connective tissue stroma of the placenta is fully formed by the end of the fourth month; in later stages, because of the intensive growth of the fetus, there is only an increase in the number of blood vessels.

SUMMARY

The author studied the development of the connective tissues in the primary and secondary chorionic villi from the earliest stages to term. He found in the former that the reticular tissue was transformed quite early into loose connective tissue in which collagen fibers came to predominate. The stroma of the terminal villi retained its reticular nature much longer. Up to the sixth week of pregnancy, the terminal villi possess both vascular and avascular types. The development of the connective tissue stroma was complete by the fourth month. Later only the number and caliber of the blood vessels increased.

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THE SENSORY INNERVATION OF THE SUPERIOR MESENTERIC ARTERY

L. S. Moroz

From the Department of Normal Anatomy (Chairman – the late Prof. G.F.Ivanov) of the First Moscow Medical Institute

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The aim of the present research has been to uncover the origin of the sensory innervation of the superior mesenteric artery.

In studies conducted by means of anatomical preparations, N. A. Kukover [7], T. V. Strukgof [8] and others have demonstrated an association of the gastric plexus with the superior mesenteric one. Also, there has been proven the association of the right vagus nerve in the formation of the superior mesenteric plexus [1, 3, 8].

The data in animals bearing on the innervation of the superior mesenteric artery is most scanty. V. M. Godinov [4] was the first to describe the sensory nerve endings within the adventitial and muscular layers of the mesenteric vessels. He indicated that these receptors lie mainly at places of branchings from the main arterial stem. T. A. Grigorieva [5] noted the presence in the arterial wall of receptors constructed, "on the general plan analogous to the one seen in the abdominal aorta" and she observed an especial plentitude of sensory receptors within the media of the artery.

By means of physiological studies, V. N. Chernigovsky [9] showed that the gastric plexus contains the main mass of the sensory fibers of the small intestine; the removal of this plexus leads to a disappearance of those reflex phenomena which are mediated by the visceral interoceptors.

V. A. Ivanov [6] found that the receptors in the walls of the mesenteric vessels carrying the afferent fibers of the depressor reflex from the superior mesenteric artery pass through the gastric plexus being incorporated in the gastric nerves.

EXPERIMENTAL METHODS

We made anatomical dissections, using histological methods of study and experiments on cats and dogs. The anatomical studies were conducted on human cadavera as well as on cats and dogs. The histological studies of the nerve fibers and their ultimate ramifications in the human superior mesenteric artery were conducted on bodies of persons dying of trauma, similar observations being made on the walls of the cat and dog superior mesenteric arteries, the method of Bielschowsky-Gross-Lavrentiev being employed as well as the Kampos procedure.

The sources of the sensory innervation of the superior mesenteric artery we determined by experimental histological procedures. The following operations were performed: removal of spinal cord ganglia in the chest and lumbar divisions; sectioning of the gastric nerves within the abdomen; excision of the ganglia of the recurrent nerve. To determine the sources of the sensory innervation of the superior mesenteric artery, we severed in cats and dogs in sequence from each side either one or two adjoining thoracic and lumber spinal cord ganglia.

The animals were sacrificed in 6 days.

The data from the anatomical preparations indicates that in man the superior mesenteric plexus is formed by branches from the gastric plexus, splenic plexus and branches from the right vagus. The nerves around the

superior mesenteric artery form a rich periarterial network from which fibers direct themselves to the vessel sheath. From this periarterial network of the main stem fibers go to the intestinal arteries as they form loops and go also to the numerous branches entering the intestinal walls. In dogs and cats the basic construction is similar but the branches are smaller and the architecture of the nerve ramifications is more simple.

Fig. 1. Typical Krause corpuscle on an arteriole in the outer sheath of the human superior mesenteric artery. Microphotograph. Magnification 600x.

The human superior mesenteric artery has numerous free nerve endings in the adventitial and muscular layers as well as special end-organs of the sensory type such as the Vater-Pacini laminated corpuscles and the Krause bodies (Fig. 1) lying in the outer sheath and in the perivascular connective tissues. We were able to demonstrate similar structures in the superior artery walls of a cat but only the free nerve endings in dogs.

EXPERIMENTAL RESULTS

Study of our histological preparations demonstrated that the nerve trunks and the nerve fibers enter the wall of the superior mesenteric artery from the side of its external sheath. Both superficially and in depth, the adventitia of the superior mesenteric artery has free nerve endings forming bushy clusters of branching nerve fibers. In addition, the outer sheath of the vessel has bush-like sensory nerve endings served by specialized cells.

Even within 48 hours following the removal of spinal cord ganglia, the nerve fiber and its sensory ending can be seen to be in the initial stage of degeneration. After 72 hours the disintegration of the nerve fiber is easy to see (Fig. 2). After 5-6 days, within the adventitial wall of the vessel the fragmentation of the fibers of the receptor nerves is sharply defined.

Our data is in agreement with that of T. A. Grigorieva [5] and they demonstrate that the media of the superior mesenteric artery has a rich supply of sensory nerve endings. In the muscular layer are bush-like free sensory nerve endings and also free nerve endings associated with the collagen fibers. At the boundary between the outer and inner vessel sheaths lie the receptor field whose endings are in the muscular layer and which are supplied with special cells. Some fields are occupied by fibers having a step-ladder appearance, their nerve fibers being associated with bundles of elastic collagen fibers. T. A. Grigorieva [5] speculates that these receptors of the muscular layer associated with the collagen fibers or lying immediately upon them serve the purpose of registering the tone of the blood vessels and the degree of blood filling.

Degeneration of the receptor endings within the outer and middle sheath of the superior mesenteric artery is caused by removal of spinal cord segments from the 5th to the 13th. It follows that the source of the sensory innervation of the superior mesenteric artery are nerve cells lying in the spinal cord at those levels.

Removal of the spinal cord ganglia in cats by us, as with other investigators [2, 11], led to degeneration of the myelinated fibers going to the sensory plates of the Vater-Pacini bodies. Bilateral section of the large

gastric nerves in the cat led to fragmentation of the myelinated nerve fibers going to the Vater-Pacini bodies which is a confirmation of previously published work [2, 10].

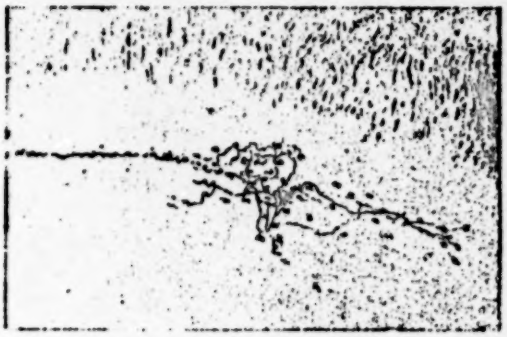

Fig. 2. Bush-like receptor ending having its own reenforcing cells lying within the outer layer of a dog superior mesenteric artery. The disintegration of the nerve fiber supplying the receptor (first stage of fragmentation). Bilateral removal of thoracic segments 6-7, 72 hours after the operation. Microphotograph. Magnification 600 x.

Fig. 3. Laminated bodies of the Krause corpuscle type in the outer covering of the superior mesenteric artery near a blood capillary. Nerve fiber is fragmenting. Removal of right fascicle of cat. 72 hours after operation. Microphotograph. Magnification 600 x.

Removal of the right as well as the left gastric ganglia of the vagus nerves in dogs and cats (the animals being sacrificed 3-6 days after surgery) caused degeneration of nerve fibers and free nerve endings within the wall of the superior mesenteric artery. After the removal of the ganglionic bundles in cats, degeneration of the axes of the myelinated nerve fibers could be observed at their entrance to the laminated bodies of the Krause type as seen within the outer sheath of the superior mesenteric artery (Fig. 3). We, just as V. M. Godinov [4], observed in the deeper layer of the external sheath of the superior mesenteric artery sensory nerve endings at places where branches left the main stem.

In the adventitia of the dog superior mesenteric artery, we uncovered polyvalent nerve endings, terminating fibrils of which lay on the vasa vasorum and in the surrounding connective tissue. Such receptors T. A.Grigorieva [5] designated as tissue-vascular and deemed them to be regulators of the metabolic intra-tissue exchanges. In the perivascular connective tissues, along the course of the nerve fibers and in the adventitia of the superior mesenteric artery we saw nerve cells lying singly or in groups.

SUMMARY

Experimental studies demonstrated that spinal ganglia from the fifth to thirteenth thoracic segments as well as both the vagi nerves partake in the sensory innervation of the superior mesenteric artery. Laminated medullary corpuscles surrounding the sheaths of the superior mesenteric artery derive their nerve fibers from the above ganglia. Krause-type corpuscles in the perivascular connective tissues have their innervation from similar sources.

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THE FUNCTIONAL STATE OF THE CHOROID PLEXUS EPITHELIUM IN THE BRAINS OF HUMANS DYING OF TUBERCULOUS MENINGITIS

G. G. Avtandilov

From the Department of Normal Anatomy (Chairman – the late Prof. A. N. Gennadiev) of the North-Ossetia Medical Institute (Director – Assistant Prof. S. N. Polikarpov), the City of Ordzhonikidze and the Pathological Anatomic Division of the Republic of Kabardin-Balkarian United General Hospital (Chief Physician – T. K. Kandaliev) at Nalchik

(Received August 24, 1956. Presented by Active Member Acad. Med. Sci. USSR I. V. Davidov)

Most investigators, in spite of the multiplicity of contradictory opinions, believe that spinal fluid is formed by the choroid plexus of the brain [3, 10, 12, 13]. Information shedding light on the functional state of these plexi in the course of various diseases in general and tuberculous meningitis in particular is quite scanty, although the pathological anatomy of the vascular plexi has been described rather fully [2, 7, 11, 14, 15].

With the aid of the double salt injection method of A. N. Gennadiev [4, 5], we were able to demonstrate on dogs the importance of the intercellular substance of the plexi in the brain for the transfer of electrolytes between the blood plasma and the spinal fluid; the cellular elements of the epithelial cover of the plexi surviving the moment of the organism's death and not taking the stain [1].

On this basis, it seems evident that we became interested in investigating the functional state of the epithelium covering the choroid plexi lining the lateral ventricles of the brain in individuals dying of tuberculous meningitis.

EXPERIMENTAL METHODS

Injections were undertaken in corpses of 22 patients dead of tuberculous meningitis and 8 individuals brought shortly after sudden violent death (control group).

The lateral ventricle of the brain was entered with a needle and 10-20 cc of a mixture composed of potassium iron blue solution (3.76%) made isotonic with blood plasma was introduced. Perfusion of the ventricles was accomplished by careful removal of the calvarium (making certain not to damage the dura mater), or by making a trephine opening in the parietal bone. Before injecting the fluid, an equivalent or somewhat lesser quantity of spinal fluid was removed. Several minutes after the above operation, 50-60 cc of a mixture of More's solution (double sulphate salt of iron and ammonia – 3.53%) made isotonic with blood plasma was injected into the homonymous carotid artery. The order of the injections of the mixtures and the time intervals between them were varied. As a result of the chemical reaction between the salts that had been introduced, insoluble Turnbull blue would precipitate in the tissues.

Following the second injection, the brain was removed and studied histologically. The choroid plexi of the lateral ventricles were fixed in 10% neutral formalin and then studied as total transilluminated prepatations and histological sections stained with hematoxylin eosin and hematoxylin-picrofuchsin. All experiments were done from 4 to 12 hours after death.

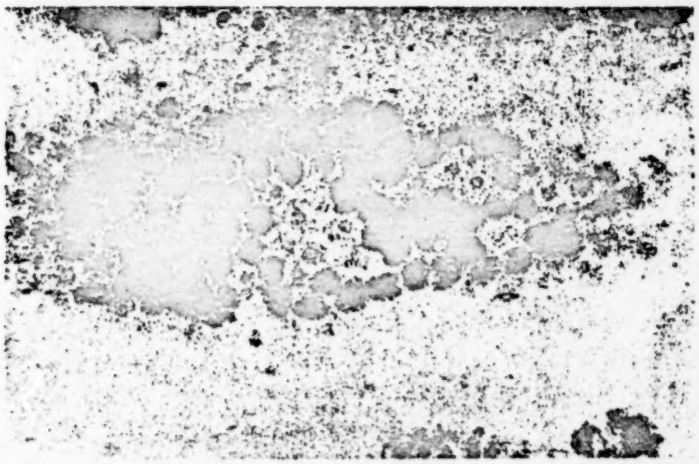
RESULTS

Microscopic studies of the villi of the vascular nets in the lateral ventricles taken from bodies of the control group within the first hours after death, in the total preparations, as well as in the histologic sections, showed the Turnbull blue to have precipitated on the surface of the cells and in the intercellular substance between the epithelium, in the connective tissue stroma, the endothelial lining of the capillaries as well as in the lumen of the vessels.

The results are analogous to those seen in the vessels of experimental animals under similar experimental conditions.

As the time interval after death was prolonged, there was seen a diffuse staining first of the surface and later of the protoplasm lying more deeply within the epithelial cells. As a rule, within 6 hours of death almost the entire epithelial plate would take the blue shading; the cellular elements taking more of the dye more evenly although some cells exhibited idiosyncracies, taking the dye more lightly.

Study of villi in patients dying of tuberculous meningitis and subjected to similar alternation of electrolytes, gave results differing in some particulars from those in the control group even when the time interval after death was the same for both.



Varying functional condition of the epithelial cells of the choroid plexus in the human brain during tuberculous meningitis. The following cells can be seen: those which do not take the Turnbull blue, those which have blue granules, and those which stain a diffuse blue. Intercellular spaces are widened. (Patient M., age 3 years, 4 hours after death; microphotograph, ocular 10×, objective 40×; hematoxylin-eosin).

In some instances, besides the usual picture of the villi in tuberculous meningitis, one could see increase in the size of the epithelial cells and of the spaces between them.

Turnbull blue was precipitated at various stages of the electrolyte transfer and lay in thick folds on the surface and between the epithelial cells. In one epithelial sheet there could be seen cells having a different response to the Turnbull blue. Some cellular elements proved to be intact: protoplasm stained evenly with eosin to a delicate rose, rounded nuclei, rich with chromatin. Among the unstained cells were cells having inclusions of blue granules within the protoplasm. Other cells held so much Turnbull blue as to stain the protoplasm blue, while some cells were colored diffusely (see Figure). As the time after death was lengthened, more cells were seen to take the stain diffusely.

D. N. Nasonov and V. Ya. Alexandrova [8, 9] have shown that intact cells will not take up the dye. Cells damaged by various agents and by death have increased absorptive capacities which permit the dye to penetrate. It follows, that the uncovering of intact cells in the epithelial cover of villi stricken by tuberculosis is proof of their vitality. Some cells are in a state of paranecrosis to which the blue granulation is proof. The epithelial cells which take a diffuse staining must have undergone far advanced alteration and death.

Tuberculous meningitis affects the epithelial cover of the vessel villi unevenly, as our observations show that the Turnbull blue is being absorbed variously, the cells being seen to be in different stages of vitality – this being reflected much more than is usually seen in the control studies.

The loosening of the epithelial cover, and the dystrophy reflected in the variable functional states of the cellular elements are probably the preconditions of the increased permeability for circulating substances carried in the bl

This state of the epithelial cover is reflected in the rise during tuberculous meningitis of the quantity of albumin present in the cerebrospinal fluid in the ventricles of the brain.

The material just presented serves as confirmation to I. V. Davidovsky who states that, "when other conditions are equal, the functional state of the organ and its tissues will determine its vulnerability" [6].

Apparently, one of the most important pathological physiologic properties of tissues is the variable functioning of the individual cells associated with the constant alternation between anabolic and catabolic processes this determining very largely their variable susceptibility to being damaged. This last would be least in cells still immature.

SUMMARY

By studying the absorption of Turnbull blue in the corpses of 22 patients dead of tuberculous meningitis and 8 individuals brought shortly after sudden violent deaths and serving as controls, the author has shown that tuberculous meningitis destroys the cells of the epithelial cover of the choroid plexus quite unevenly: some cells necrotize and die while others are still quite viable. The metabolic variation of individual cells has an important influence in determining their susceptibility at the time of being subjected to toxic influences.

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NEW METHODS

TWO METHODS OF ISOLATED BILATERAL DESTRUCTION OF SUBCORTICAL STRUCTURES NUCLEUS CAUDATUS AND PUTAMEN

B. N. Klosovsky, N. S. Volzhina and V. P. Kukushkina

From the Pediatric Institute Acad. Med. Sci. USSR (Director – Corresponding Member Acad. Med. Sci. USSR Prof. O. D. Sokolova-Ponomareva) and the Neurological Institute Acad. Med. Sci. USSR (Director – Active Member Acad. Med. Sci. USSR Prof. N. V. Konovalov)

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A model of bilateral isolated destruction of the nuclei of the striate system may be essential for the study of their function which to this day has not been fully clarified. Without such a model it is quite impossible to know the character of the interrelationships existing between the cerebral hemispheres and the nearest subcortical structures – the striate system.

Cases in which the striate nuclei become involved in pathological processes terminating fatally are not of value in answering the question put above. The subcortical nuclei and especially the striate system have wide compensating capacities: the functions of the excluded nucleus of the one side of the brain can be replaced by the action of the same nucleus of the other side. To determine the function of these nuclei one must have complete bilateral ablation by the pathological process. This pathological process has to either destroy them fully or else destroy their function by way of stimulation.

Clinically one sees destruction of either a portion of one or the other striate nucleus or else, when they are completely destroyed, there is also involvement of adjacent tracts and nuclei including also cerebral hemisphere damage.

Disease of one of striate nuclei – globus pallidus – is associated with appearance of compulsive movements – hyperkineses, thus there is ascribed to this nucleus a participation in the function of movement. Two other nuclei of the striate system: nucleus caudatus and putamen – are thought to exert an inhibitory effect upon the globus pallidus.

I. P. Pavlov indicated how important the "near subcortex", as he called it, was for the proper functioning of the cerebral hemispheres. However, it is not clear to this day precisely what role these nuclei play in the processes of the highest nervous activity.

To the present there has not existed a method for having a model in animals where the subcortical structures would be ablated without simultaneous damage to the cerebral cortex.

We were able to develop two methods based on different principles. These methods permit total ablation of the nucleus caudatus bilaterally without damaging the substance of the cerebral hemispheres.

One method depends on surgical extirpation of the nucleus caudatus. The other method permits the removal of the nucleus caudatus or the putamen by means of clipping or coagulating the blood vessels supplying these structures.

EXPERIMENTAL METHODS

1. Bilateral extirpation of the nucleus caudatus surgically*

* B. N. Klosovsky and N. S. Volzhina, Pediatric Institute Acad. Med. Sci. USSR.

Puppies ages 2 to 5 months or dogs ages 6 to 7 months were placed as a preliminary on a dry diet. The animals were subjected to morphine-Barbamyl narcosis. The incision was made through the skin of the head in the midline from the frontal notch to the occiput. In the posterior frontal and anterior parietal region the periosteum was scraped and rongeur forceps used in the indicated region to make a trephine opening 2.5 x 2.5 cm in size. Bone bleeding was checked by use of surgical wax.

The special nature of this operation is that the bone of the skull is removed above the longitudinal venous sinus by crossing our midline incision for 3-4 mm. The greater part of the trephine opening 2.5 x 2 cm is on the side where the operation is to be performed (in our procedures, the right).

The dura mater was incised in such a manner that the base of the flap was at the longitudinal sinus. On the two parietal veins, as they entered the longitudinal sinus or a couple millimeters prior to that, in the dura mater there were placed ligatures with subsequent coagulation with the tip of an electrocautery. Then, a broad retractor was used to push the left cerebral hemisphere to the left until the corpus callosum came into view along with the vein of the corpus callosum which traverses it. The corpus callosum was then incised longitudinally, somewhat to the right of the midline. Such an incision gives entry to the right ventricle. A curved spatula was used to elevate the upper wall of the ventricle, this permitting the sight of the head and body of the caudate nucleus.

Fig. 1. Frontal section of puppy brain. Both caudate bodies have been removed by means of this operation.

The substance of the caudate body was scooped out by use of a bone spoon, the depth of the ventricle being lighted by a special lamp. The experimenter removing the caudate nucleus used a lens magnifying 2.5x during this stage of operation. By using care, the fibers of the internal capsule were left undamaged. The slight bleeding occurring during the scooping out operation was readily checked by means of pledgets of cotton soaked in warm saline solution. Having removed the caudate nucleus from the right side, the left caudate nucleus was extirpated by an analogous procedure. At the end of the operation the portion of the brain exposed by the trephine opening was covered by a fibrin sheet, this minimizing scar formation. The flap of the dura mater was then sewed over this sheet. The animals survived this operation well, and could then be followed for years later as a long-term experiment.

Figure 1 shows the total ablation of the caudate nuclei with preservation of the fibers of the internal capsule resulting from this operation.

2. Removal of nucleus caudatus or putamen by means of application of clips or coagulation of arteries supplying these structures •.

In order to coagulate the basal arteries supplying the nucleus caudatus and putamen we used the route introduced into experimental animal surgery where the side (temple) approach is utilized in the dog. The skin incision is made half way between the eye and ear of the dog, along the temporal muscle. This muscle is incised on a slant down to the bone. The skull arch and coronary process of the upper jaw were removed. The edges of the temporal muscle were spread with a Jansen retractor, thus exposing the temporal surface of the skull. In this region the bone was trephined, baring the parietal frontal region of the brain above the Sylvian fissure. The dura mater was then opened. The brain was elevated with a spatula exposing the olfactory tract with the sulcus endorhinalis which served as an orientation marked during further surgery. Our investigations have established that the arteries supplying the nucleus caudatus, putamen and internal capsule enter the brain in this sulcus endorhinalis (branches of cerebri mediae) or just below it (branches cerebri anterior). When this part of the brain was exposed as described, either group of arteries or both could be coagulated, a magnifying lens being used.

• B. N. Klosovsky and V. P. Kukushkina, Neurological Institute Acad. Sci. USSR.

Fig. 2a. Basal area of the surface of the large dog brain. The dotted lines enclose the area where the striate arteries were coagulated as they entered the brain substance.

Fig. 2b. Frontal section through puppy brain in animal subjected to coagulation of the striate group of arteries. The caudate nucleus underwent necrosis. The blood vessels of the brain were injected with India ink. The dotted line outlines the disappearance of the necrotized nucleus caudatus.

When the appropriate arteries were surgically electrocoagulated the nucleus caudatus and the putamen underwent necrosis. This fact we established by numerous studies made histologically employing the Nissl stain and injecting the brain vessels with India ink. In the latter instance one could observe an alteration in the character of the filling of the subcortical structures being studied as compared with adjacent brain structures (white substance of the frontal region and the corpus callosum). In the caudate and lenticular nuclei were found areas of the vessel net which did not fill.

Figures 2a and 2b outline the location at the base of the brain of the arteries supplying the subcortical nuclei in the region of the olfactory cortex which were subjected to obliteration and coagulation. The sections demonstrate quite well the degeneration of the nucleus caudatus that ensues following the shutting off of the arteries feeding it.

SUMMARY

The authors described two procedures for ablation of the nucleus caudatus and putamen in such a manner as not to injure any of the surrounding structures.

One method involves surgical removal. The other procedure employs destruction of the blood supply of these nuclei.

AN AUTOMATIC TRANSMITTER OF CONDITIONED AND UNCONDITIONED STIMULI FOR DEVELOPING CONDITIONED REFLEXES IN SMALL ANIMALS

N. M. Papyshev

From the Department of Normal Physiology of the Naval Military Academy

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The process of developing conditioned reflexes in small laboratory animals (mice, rats and others) because of their biologic peculiarities encounters many practical difficulties.

Our method of approach employing the moving food method proposed by L. I. Kotlyarev in 1947-1948 [1], with the additional pneumatic record as proposed by L. Yu. Izergina and V. K. Fadeev (1949), has shown that, in order to develop rapidly conditioned reflexes in rats, one must maintain a very strict continuity in the giving of the stimuli both as to the timing and their order.

To observe these rules is somewhat difficult as it is necessary simultaneously to observe the animal's behavior and open and close switches producing on the second the conditioning and unconditioned food stimuli besides noting the character and kind of recording.

To obtain maximal objectivity in the conduct and recording of the experiment, it is necessary to automatize the entire process after having developed a stereotype. With this in view, we propose some automatic recorders and stimuli which will make the work of the experimenter simpler.

The existing apparatus for developing reflexes automatically is either difficult to make (E. A. Ganike, 1935; V. K. Fedorov, 1951) or includes only the recording of the experiment (L. I. Kotlyarev, 1951). Taking into account the merits of automatization and recording of an experiment, we developed and made an automatic instrument the nature of which is shown in Fig. 1.

The automatic transmitter acts on the principle of periodically opening and closing the electric circuit of a synchronized motor thus assuring adequate delivery of electric impulses on time.

Using the ordinary motor with two revolutions per 1 minute and employing a six gear 1:4 ratio we were able to make the drum revolve with a speed of 1 revolution in 2 minutes.

On the drum are 3 discs, each of which has 2 interacting plates, these permitting a change in the width of the teeth or distance between them.

Periodic electric impulses reach the step finder unit ATS (From a dial telephone) – see Fig. 2, with which is associated the transmission of the conditioned and unconditioned stimuli.

The step finder ATS has three movable commutator bars each of which corresponds to 12 immovable commutator bars; the first may simultaneously be relayed from one of the immovable commutators to others in conformity with the transmission of electric impulses to the coil of the step finder; moving bar I switches in the conditioned stimulus, bar II – unconditioned stimulus, while bar III alternately switches in 2 simple relays which assure the recording of the conditioned stimulus and the subsequent introduction of the conditioned stimuli into the stereotype of the experiment.

The first disc has 2 teeth and with 1 rotation of the drum the electric circuit to the step finder is closed twice as the moving commutator bar closes twice on the succeeding immovable bars.

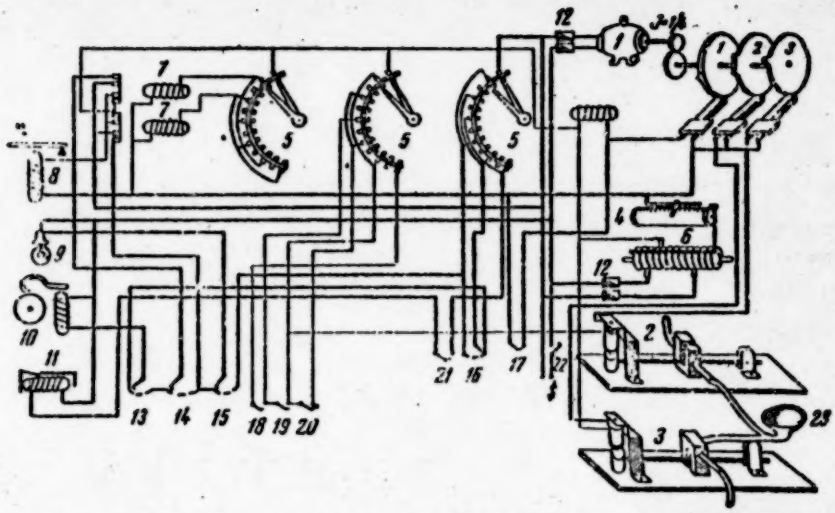

Fig. 1. Diagram of automatic producer of conditioned and unconditioned stimuli for producing conditioned reflexes in small animals.
1) motor 1270 2 turns/minute; 2) electromagnetic presentation of milk to the feeder; 3) electromagnetic release of milk from feeder; 4) sliding rheostat; 5) step finder unit ATS; 6) selenium regulator; 7) relay; 8) recorder of the conditioned stimulus; 9) electric lamp; 10) electric bell; 11) buzzer; 12) safety device; 13, 14, 15, 16) two-contact commutator; 17) electric button; 18, 19, 20) disconnecting switches; 21, 22, 23) feed container.

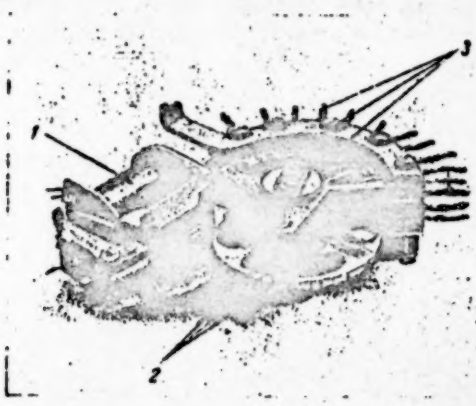

Fig. 2. Step finder ATS.
1) coil; 2) movable commutator bars; 3) immovable commutator bars.

The first time the circuit closes on the conditioned stimulator and its recording device as well as switching in the electromagnet to the feeder thus assuring the delivery of the unconditioned stimulus; finally the electric circuit is closed by the disc the width of whose teeth regulates the amount of the food stimulus; and its removal upon the giving of the conditioned stimulus being dependent on the angle between the first tooth in disc 1 and the tooth in disc 3.

In case of a negative conditioned stimulus, the electromagnet giving the unconditioned stimulus is not switched into the feeder.

The second impulse excludes the various instruments as the moving commutator bars switch into the succeeding immovable bars which are outside the electric circuit.

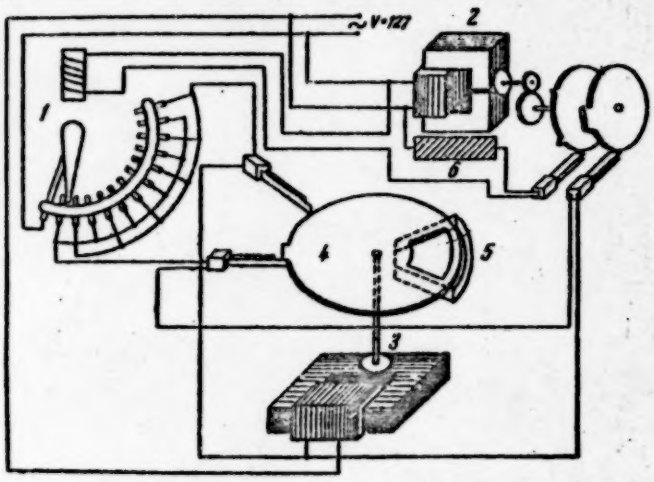

Fig. 3. Diagram of the principle of the electric feeder.
1) step finder unit ATS; 2) motor assuring delivery of impulses; 3) motor assuring working of feeder; 4) disc opening and closing feeder; 5) feeder.

From the relay commutator we can obtain conditioned stimuli in the needed sequence. We used the stereotype light-bell-buzzer (differentiating to the bell), corresponding to this, the immovable bars were set to light-bell-buzzer-light-bell-buzzer but, it is possible to get a stereotype having two, three, four or six signals.

For feeding, the diagram shows a direct current introduced with a selenium rectifier while the needed strength is assured by a constant resistance which is determined empirically.

Re-enforcement of the conditioned stimuli (in our case milk) is also done automatically with the aid of two electromagnets which automatically open or close the tubes bringing the milk to or taking it away from the feeder in correspondence with the appropriate conditioning signals.

The tube bringing the milk is always clamped and milk can flow by gravity into the feeder only when the electromagnet switches in, this being done by simultaneous closing of circuit to moving commutator bar II and disc 3.

The hose leading away from the feeder is clamped during the re-enforcement period, thus permitting the milk to stay in the feeder, this being accomplished by the other electromagnet which is thrown into the circuit through disc 2. This transmitter permits electric or pneumatic feeding thus permitting use of either food stimulus. The principle is given for electric feeding stimulus in Fig. 3.

For subsequent alterations of the stereotype, for changing the reflexes and for introducing the step finder into the base conditions there is shown a switch-board which contains 4 commutators, 5 current breakers and 1 button (see diagram).

Use of the switch-board permits any combination of three signals.

Actual use of this automatic transmitter has shown that it aids greatly in the task of developing conditioned reflexes. The usual city current activates it thus obviating the need for a steady source of direct current

and its standard details are quite simple: motor, step finder ATS and selenium rectifier. This automatic transmitter assures a correct continuity and sequence of signals at the predesignated time intervals. The transmitter permits the setting up of a stereotype composed of several signals and guarantees errorless re-enforcement.

SUMMARY

We developed an apparatus which will permit automatic transmission of conditioned and unconditioned stimuli for the development of conditioned reflexes in small animals. The step-finder, the synchronous motor, the switch board and all the numerous details are well-illustrated.

This transmitter facilitates laboratory investigations and permits a very objective and precise approach to the animals. It may be connected either to electric or pneumatic feeding devices and may be used in standard conditioned reflex boxes.

EXPERIMENTAL BRONCHOPNEUMONIA

A. E. Ivanov and V. F. Sosova

(Scientific Directors: Corresponding Member Acad. Med. Sci. USSR
Prof. N. A. Kraevsky and Prof. N. N. Klemparskaya)

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In the investigation of pneumonia to the present day there exist many unsolved questions. One of the reasons for this is the absence of a reliable method by means of which experimental pneumonia can be produced so that the natural course of the disease could be duplicated.

Most workers attempted to reproduce croupous pneumonia, using as a basis the allergic character of the illness [1, 2, 4, 5, 10, 14, 15, 16, 17, 18, 19].

When attempts were made to reproduce bronchopneumonia, the successes were few. The attempts in this direction can be divided into two groups.

In the first group are experiments affecting the various divisions of the nervous system which upset the circulation by reflex means and so produce a consequent pneumonia. Thus, A. D. Speransky [10] established that irritation in the subcollicular regions of the brain leads to trophic lung disturbances against which background pneumonia may develop. A. V. Tonkikh [11] described pneumonia following crushing of cervical sympathetics (in cats) or sectioning of vagi (in cats and dogs). Analogous results were obtained by A. M. Chernukh by injecting turpentine along neural sheaths of vagi (in rabbits) [13] and I. P. Dolgachev [3] when applying radon to the upper cervical sympathetic node (rabbits, cats). However, many of the above experiments failed to have adequate pathologic and microbiologic controls.

As was shown by B. I. Monastyrskaya and Ya. Ya. Sokolskaya [6, 7, 9] action on the vegetative nervous system disturbs the circulation in the lungs which can exist a long time without producing inflammatory changes. Only in occasional instances with bilateral vagal section or stimulation of the sympathetic nodes will aspiration pneumonia be an end result.

In the second group of models are attempts to reproduce pneumonia by affecting lung tissues directly by way of intratracheal infection of the animals with live bacterial cultures. This was the method employed by Z. L. Golsand and A. V. Tsinzerling [12] on rats and D. S. Sarkisov and L. Ya. Ebert on cats [9]. The first infected rats with Friedlander bacilli in a 6% mixture with gum arabic. The microbial culture was introduced into the animal tracheal lumen, first incising the overlying skin while the animals were under ether narcosis. As a result the animals developed lobar or large-focus bronchopneumonia In their experiments there was a combination of bacteriologic and morphological studies.

However, this method has been successful in rats only, and then only if Friedlander bacilli are used. This is a limiting factor when using the method of Golsand and Tsinzerling.

D. S. Sarisov and L. Ya. Ebert introduced intratracheally into cats under ether and chloroform narcosis cultures of Staphylococcus aureus in a 16% suspension of polyvinyl alcohol. This alcohol was chosen for the prolonged fixation and more even distribution of the organisms through the lungs. In animals infected by this procedure isolated small foci of pus inflammation arose around the bronchi.

A serious inadequacy of both of the just described methods is the use of gum arabic, polyvinyl alcohol and inhalation narcosis. These substances undoubtedly have an additional injurious effect upon the lung tissue so that the pneumonia produced is in a large degree quite different from those occurring spontaneously.

Taking all the above into consideration, in 1954 we worked on rabbits attempting to produce pneumonia by intratracheal injections of cultures of B. enteritidis gartneri the original culture having been recovered from a spontaneous case developed by a rabbit.

The cultural characteristics of the microbe are indicated in the table.

Morphology	Motility	Biochemical response						Titer of agglutination reaction with sera				Lethal for mice
		Glucose	Lactose	Mannose	Saccharose	H_2S	Indole	Abdominal typhus	Paratyphoid B.	Breslau	Gartner	
Gram-negative rod	+	KG	0	KG	0	+	0	1:1,600	1:800	1:200	1:6,400 1:12,800	+

EXPERIMENTAL METHODS

Intratracheal introduction of the microbes was performed by using a needle without any previous incision or narcosis. The animals withstood the manipulations quite well. First the skin on the anterior surface of the neck was clipped and shaved as thorougly as possible. The rabbit was then bound to a board so that the head fitted into a hollow in its upper portion while the fore paws could be spread as far apart as possible and also downward. The skin of the area to be injected was sterilized with iodine and alcohol. The board was tilted at about 40-45° of head elevation and the head was also tilted backwards. In this position it was a simple matter to palpate the cartilage of larynx and trachea. The skin was first pierced by the needle held in exactly the midline. Then the fingers of the left hand fixed the trachea which was pierced by the neeedle about 0.5-1 cm below the larynx. At the moment of entry which was always from above down the needle was held at a maximal angle from the surface of the skin. This was done to avoid injuring the posterior surface of the trachea. The puncture was done with a sterile needle attached to a hypodermic syringe half filled with physiological saline so as to avoid infecting any surrounding tissues at the moment of entry.

After the puncture we made certain that the needle tip was in the tracheal lumen. This was readily confirmed by pulling back on the piston of the syringe and obtaining air bubbles. Then the syringe holding the saline was replaced with the one having the required amount of infecting material, and the material was introduced quite slowly so as to minimize a possible cough reflex.

The infecting material was a suspension of B. enteritidis gartneri in physiological saline. From a one day agar culture a mixture was prepared with 100 million microbes per $^1/_2$ cc of physiological suspension (using optical bacteriological standards). As indicator which was to aid in judging the spread of the suspension through the lungs, Trypan blue was added (0.5% suspension). Experiments proved that this addition did not affect the pathological alterations.

EXPERIMENTAL RESULTS

Of 20 rabbits infected by the above method in the final confirmatory series, all developed pneumonia. One only died on the 2nd day after being infected; 11 rabbits were sacrificed on the 7th day; 1 died on the 17th; 4 were sacrificed on the 20th day and 2 — on 30th day after the infection.

Daily blood cultures taken from the rabbit ear veins were consistently negative. This proved that the quantity chosen by us was sufficient to produce infection but did not produce bacteremia.

At autopsy all the animals were seen to have extensive pneumonic areas, sometimes involving an entire lobe or almost an entire lung (Fig. 1).

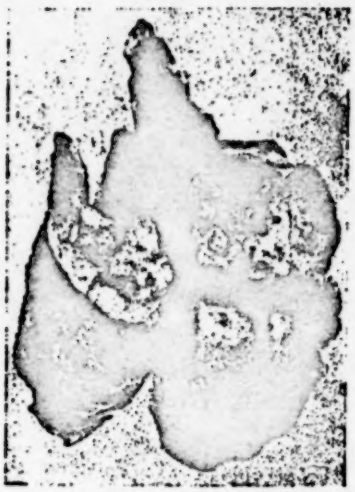

Fig. 1. Extensive areas of pneumonic involvement in both lungs 7 days after introduction of the infection.

The affected lung was much increased in volume, solidified, red in the first days, later mottled with yellow-grey areas and, when bluing was employed, of a bluish grey color (Fig. 2). The sections of the affected lung tissues had the same colorations and a finely granular surface from which steamed a thick, cloudy fluid.

The microscopic picture was that of bronchopneumonia (Fig. 3).

When the animal died in the first days after being infected, the alveoli had a serous exudate with an admixture of erythrocytes. Later, by the 7th day, the foci of consolidation had many alveoli and small bronchioles filled with exudate having pseudo-eosinophilic leucocytes, macrophages, septal cells and erythrocytes. In places, fibrin strands could be seen. Within the exudate the microbes were easy to see as they stained intensely with hematoxylin and were principally extracellular. Most of the cells within the exudate were in a state of necrobiosis and fragmentation. In the protoplasm of the macrophages were seen fragments of nuclei and eosinophilic granulation from the disintegrated leucocytes, microbes and granules of Trypan blue. The presence of the bluing in the alveoli indicated that the microbes introduced into the trachea had been able to reach the alveoli directly. Within the bronchi, the exudate consisted principally of leucocytes, cells of bronchial epithelium, macrophages, erythrocytes and microbes all mixed together with mucus. In the peribronchial tissues were observed lymphangeitis and hyperemia of the peribronchial lymph nodes. In places, within the lumen of the

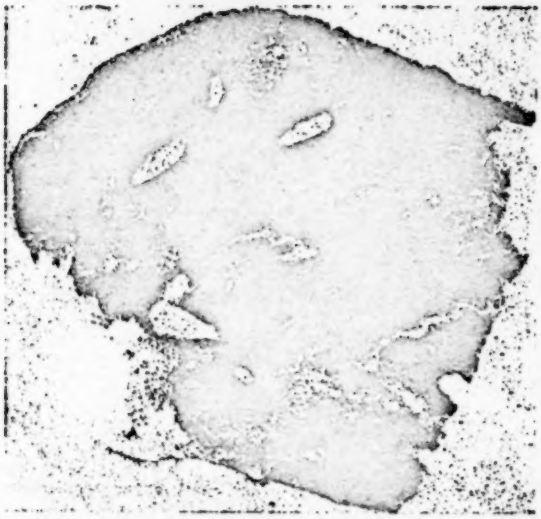

Fig. 2. An area of bronchopneumonia. Total section through the lower lobe of a lung on the seventh day after infection (stained with hematoxylin-eosin).

lymph vessels and within the lymph nodes, there were seen granules of the Trypan blue. In addition, there were lung areas with evidences of disturbed circulation (hyperemia, stasis, erythrocytic diapedesis with perivascular and peribronchial hemorrhages, edema of the stroma and alveoli).

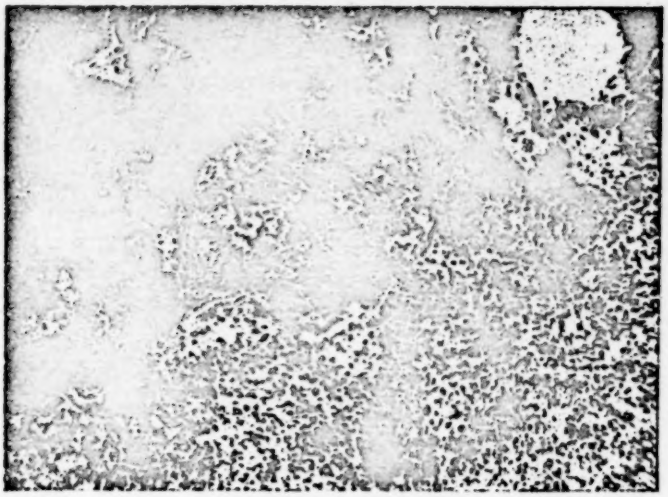

Fig. 3. Total section through the lower portion of the lung on the seventh day after infection. Alveoli of the lungs are filled with a catarrhal exudate with a large admixture of leucocytes; peribronchitis (hematoxylin-eosin stain, high magnification).

By the 17th-20th day after the infection, the pneumonic areas were much smaller than at the height of the infection on the 7th day. Microscopically, there were seen areas of atelectasis, a moderate number of leucocytes and many macrophages in the exudate. By the 30th day the inflammatory residues in the lungs were inconsequential.

All specimens taken at autopsy from the heart, spleen and gallbladder with one exception only were sterile. The rabbit that died on the 2nd day had pure culture of B. enteritidis gartneri recovered from specimen taken from its spleen. Alongside of this, all rabbits sacrificed between the 7th and 20th days had demonstrable bacteria in the lungs. By the 30th day the bacteria could no longer be obtained from the lungs either.

Thus, we have developed a completely reliable method for producing in rabbits a bronchopneumonia not associated with introduction of foreign bodies (such as plastic substances, gum arabic and agar) or the use of narcosis, not provoking bacteremia and assuring an adequate life span for the infected animals. Finally, our method does not demand the use of an unusual strain of microbe. The B. enteritidis gartneri we used is seen in spontaneous pneumonias and, therefore, is obtainable for experimental purposes.

SUMMARY

An experimental method for producing pneumonia in rabbits is presented. A culture of B. enteritidis gartneri was obtained from a rabbit having developed the disease spontaneously. This organism was used to reproduce the disease in 100% of the animals inoculated intratracheally.

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• In Russian.

•• Figure obscured in original text.

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March, 1958

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[Biological Sciences]

Abbreviation*	Journal*	Translation
...iol.	Agrobiologiia	Agrobiology
...sherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
...tibiotiki	Antibiotiki	Antibiotics
...rmacevt. Delo	Aptechnoe Delo	Pharmaceutical Transactions
...kh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
...kh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
...kh. Patol.	Arkhiv Patologii	Archives of Pathology
...Biofizika	Biofizika	Biophysics
...Biokhimiia	Biokhimiia	Biochemistry
...Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
...Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
...Biul. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
...Biul. Moskov. Obshchestva Ispytatelei Prirody. Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
...Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
...Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
...Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
...Farmatsiia	Farmatsiia	Pharmacy
...Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
...Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
...Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
...Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
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...Khirurgiia	Khirurgiia	Surgery
...Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
...Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
...Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
...Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
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...Mikrobiologiia	Mikrobiologiia	Microbiology
...Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
...Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
...Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
...Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
...Pediatriia	Pediatriia	Pediatrics
...Pochvovedenie	Pochvovedenie	Soil Science
...Priroda	Priroda	Nature
...Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
...Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
...Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
...Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
...Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
...Stomatologiia	Stomatologiia	Stomatology
...Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
...Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratorii	Transactions of the Helminthology Laboratory
...Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologii	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologii	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologii, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimicheskii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimii	Uspekhi Biokhimii	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologii	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologii	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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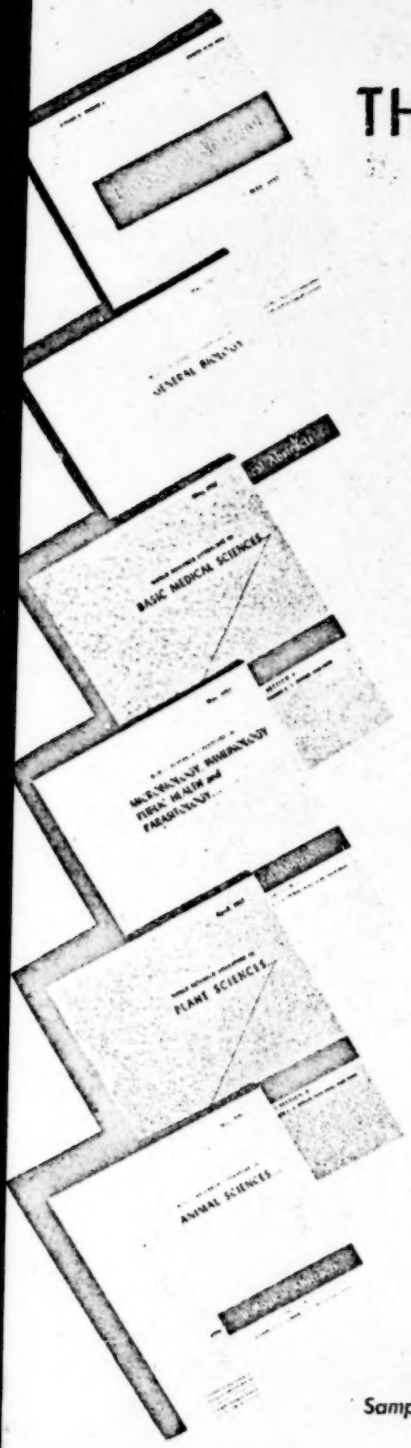

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